JOURNAL OF VIROLOGY, Mar. 1992, p. 1774–1776
0022-538X/92/031774-03$02.00/0

Vol. 66, No. 3

A Point Mutation within a Distinct Conserved Region of the Herpes Simplex Virus DNA Polymerase Gene Confers Drug Resistance

CHARLES B. C. HWANG, KATHERINE L. RUFFNER, AND DONALD M. COEN*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

Received 26 September 1991/Accepted 5 December 1991

We have shown that a drug-resistant mutant from a clinical isolate of herpes simplex virus contains a single point mutation in the DNA polymerase gene that confers resistance to both acyclovir and foscarnet. The mutated amino acid is located within a distinct conserved region shared among α-like DNA polymerases which we designate region VII. We infer that these conserved sequences are directly or indirectly involved in the recognition and binding of nucleotide and PP_i substrates.

Herpes simplex virus (HSV) DNA polymerase (Pol) is both an important target for antiviral therapy and an excellent model for the α-like DNA polymerases (reviewed in reference 6). HSV DNA Pol contains several regions, designated I through VI, conserved among α-like DNA polymerases (20). Sequence analysis of several HSV *pol* mutants revealed that mutations that confer resistance to antiviral drugs that mimic and/or compete with deoxynucleoside triphosphate (dNTP) or PP_i are distributed within regions I, II, III, and V and in region A, which is shared by only certain α-like DNA polymerases (Fig. 1B). This suggests that these conserved regions are directly or indirectly involved in recognition and binding of nucleotides and PP_i (reviewed in reference 6). Here we report the sequence of a *pol* mutant derived from a clinical isolate that implicates a distinct conserved region, which we term region VII, in these functions.

Sacks et al. (17) previously reported a heterogeneous HSV type 1 isolate from an immunocompromised patient who had suffered severe progressive herpetic disease despite acyclovir (ACV) therapy. This isolate included a mutant, termed 615.8. A marker transfer experiment in which a 3.3-kbp *Bam*HI fragment from the 615.8 *pol* gene transferred foscarnet (PFA) and ACV resistance to genetically related pretreatment isolate 294.1 or laboratory strain KOS established that the 615.8 *pol* gene contains a mutation(s) that confers resistance to both PFA and ACV (17).

Finer mapping of the 615.8 drug resistance mutations. To localize the PFA resistance marker of 615.8 more finely, a marker transfer experiment was performed as described previously (5). Three *Kpn*I subfragments from plasmid p615.8BQ (referred to as 615.8 3.3-kbp *pol* in reference 17) containing the 3.3-kbp *Bam*HI *pol* DNA of 615.8 were used (Fig. 1A). Two of the subfragments were similar in size (1.1 kbp) and extended from the *Kpn*I site of the pUC19 vector to the second *Kpn*I site within the 3.3-kbp *pol* DNA insert and were isolated together from an agarose gel. The third fragment (3.7 kbp) included the remaining sequences of the *pol* insert, extending from the second *Kpn*I site, and also included sequences of the cloning vector (Fig. 1A). This 3.7-kbp *Kpn*I fragment transferred PFA resistance to infectious KOS DNA at a frequency (0.12%) substantially higher than that obtained when no fragment (0.0047%) or the 1.1-kbp *Kpn*I fragments (0.002%) were added.

PFA-resistant recombinant virus (615.8 P^rK1) from the marker transfer experiment using the 3.7-kbp *Kpn*I fragment was then plaque purified and tested for sensitivity to ACV. Both the original resistant isolate, 615.8, and the recombinant virus, 615.8 P^rK1, were similarly resistant to ACV with a 50% effective dose of 40 μM, while KOS exhibited a 50% effective dose of 6 μM (Fig. 2). Since the corresponding DNA fragments derived from pretreatment isolate 294.1 failed to transfer the PFA resistance marker to KOS (17) and those fragments from 615.8 did transfer the resistance marker to both 294.1 and KOS (17; this report), these results localized the PFA and ACV resistance markers of the 615.8 isolate to the 1,125 bp between the *Kpn*I and *Bam*HI sites of the 615.8 DNA *pol* gene.

Identification of the mutated amino acid that confers PFA and ACV resistance. The 1,125-bp *Kpn*I-*Bam*HI regions of both 615.8 and pretreatment isolate 294.1 were then sequenced on double-stranded plasmid DNA by using the M13 universal primer (Promega Biotec) and *pol*-specific oligonucleotide primers (10) and Sequenase (U.S. Biochemicals) as recommended by the manufacturers. The region of the 615.8 *pol* gene contained a single change of C to T at nucleotide 2821, relative to that of 294.1. This transition mutation converts tyrosine to histidine at amino acid residue 941. We conclude that the change at amino acid 941 is the mutation responsible for the resistance of 615.8 to both PFA and ACV. In addition, both 294.1 and 615.8 contained two nucleotides different from those of the wild-type strain KOS sequence. One of these, an A-to-G change, generated a methionine-to-valine change at position 905. However, both nucleotides are identical to those of the HSV strain 17 sequence (16).

The 615.8 mutation alters a highly conserved tyrosine within region VII of sequence similarity. Amino acid 941 is not located within any of the five conserved regions (I, II, III, V, and A) previously shown to mutate to confer drug resistance (reviewed in reference 6) nor in the other conserved regions identified by Wong et al. (20). However, amino acid 941 is part of a motif recognized by several investigators and termed variously region 4, 9, or IX (3, 13, 18). This motif is illustrated in Fig. 1B and contains a highly conserved sequence, Lys-Lys-X-Tyr, with the second Lys and the Tyr conserved in all of the polymerases examined. Remarkably, it is this Tyr that is altered in 615.8. The first Lys is

* Corresponding author.

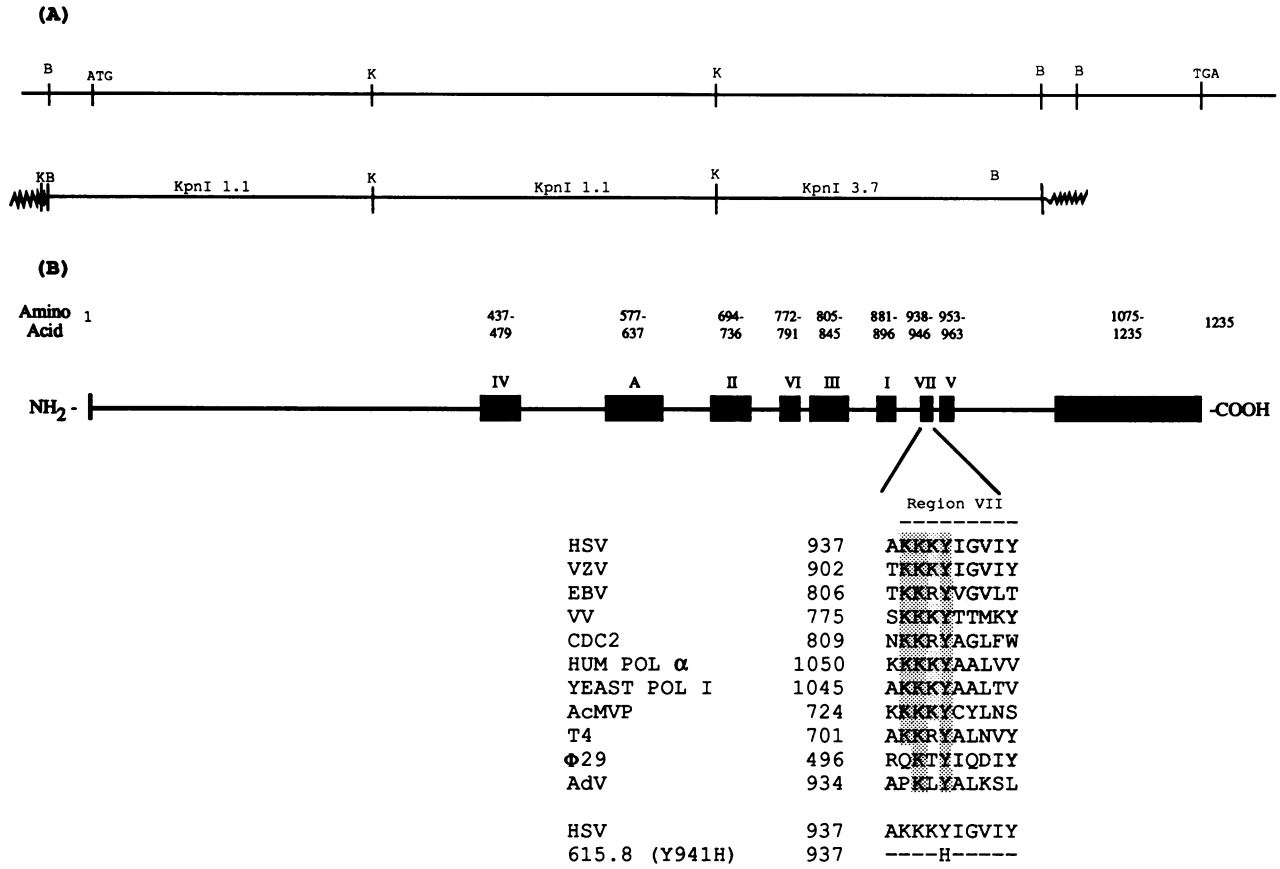

FIG. 1. (A) p615.8BQ relative to the HSV *pol* locus. The top line represents the HSV *pol* locus, with relevant restriction enzyme sites and the locations of the initiator methionine codon ATG and the termination codon TGA shown. The second line represents the DNA fragments used in this study. The 3.7-kbp *Kpn*I fragment also contains the plasmid cloning vector pUC19 sequences (wavy line). (B) Region VII of sequence similarity among α-like DNA polymerases. The top line shows a schematic of the predicted HSV Pol polypeptide, with the locations of sequence similarities indicated. Below are shown the amino acid sequences of region VII from various DNA polymerases. The following polymerases are represented: HSV (11); varicella-zoster virus (VZV) (7); Epstein-Barr virus (EBV) (1); vaccinia virus (VV) (8); *Saccharomyces cerevisiae* Pol δ (CDC2) (4); human Pol α (HUM POL α) (20); *S. cerevisiae* Pol α (YEAST POL I) (15); baculovirus Pol (AcMVP) (19); T4 DNA polymerase (T4) (18); bacteriophage φ29 polymerase (φ29) (21); and adenovirus type 2 DNA polymerase (AdV) (12). The conserved amino acids shared among HSV and the other polymerases are shaded.

conserved among all of these DNA polymerases except those of bacteriophage φ29 and adenovirus type 2. To be consistent with the nomenclature of Wong et al. (20), we propose that this motif be designated region VII.

Conserved region VII function in substrate recognition and binding. A mutation such as that of 615.8, which confers resistance to PFA and ACV, is expected to alter an amino acid involved in recognition of the PP_i and nucleotide substrates that PFA and ACV mimic. This suggests that region VII is involved in recognition of these substrates. However, there are not enough data to indicate whether region VII directly participates in substrate recognition or whether its involvement is more indirect. Region VII thus joins conserved regions I, II, III, V, and A as a site for drug resistance. This adds to a model proposed previously in which interactions among various regions might form binding sites for PP_i, dNTPs, and drugs (10).

Secondary-structure analysis by Blanco et al. (2) predicted that region VII, including the highly conserved sequence Lys-Lys-X-Tyr, had an α-helix structure while hydrophobic residues farther downstream were considered to favor dNTP binding. This was partly based on an attempt to align

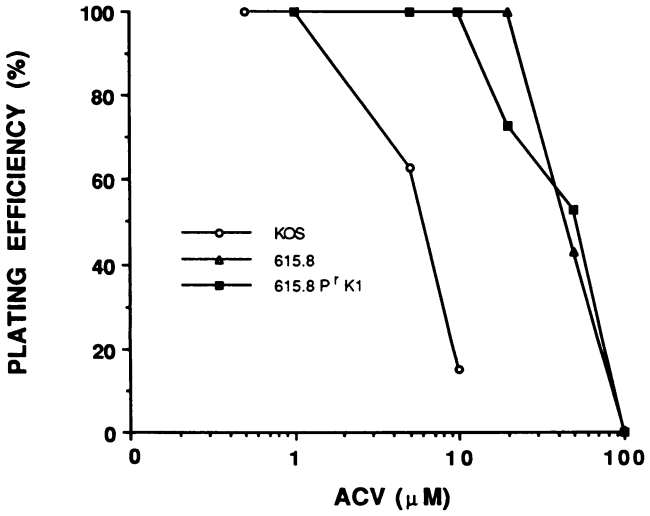

FIG. 2. Effect of ACV on plaque formation by wild-type KOS, mutant 615.8, and recombinant 615.8 P^rK1.

sequences between φ29 Pol and the crystal structure of *Escherichia coli* PolI (14). On the other hand, secondary-structure predictions by others (13) noted a structure transition from α-helical to β-sheet at the third amino acid of the motif (Lys-940 in HSV Pol). This transition is shifted to amino acid 941 (His) in the 615.8 Pol, on the basis of the protein secondary-structure analysis of Garnier et al. (9). Therefore, it is possible that the Tyr-to-His change could alter the secondary structure of this region of Pol to alter recognition and binding of drugs. Determination of the structural consequences of the Tyr-to-His change will, however, require further biophysical and biochemical studies of α-like DNA polymerases.

We thank T. Wang for helpful discussions and blessing in designating the Lys-Lys-X-Tyr motif as region VII.

This work was supported by NIH grants AI19838 and RR05381.

REFERENCES

1. **Baer, R., A. T. Bankier, M. D. Biggin, P. L. Deininger, P. J. Farrell, T. J. Gibson, G. Hatfull, G. S. Hudson, S. C. Satchwell, C. Seguin, P. S. Tuffnell, and B. G. Barrell.** 1984. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. Nature (London) **310:**207–211.
2. **Blanco, L., A. Bernad, M. A. Blasco, and M. Salas.** 1991. A general structure for DNA-dependent DNA polymerases. Gene **100:**27–38.
3. **Blasco, M. A., L. Blanco, E. Pares, M. Salas, and A. Bernad.** 1990. Structural and functional analysis of temperature-sensitive mutants of the phage φ29 DNA polymerase. Nucleic Acids Res. **18:**4763–4770.
4. **Boulet, A., M. Simon, G. Faye, G. A. Bauer, and P. M. J. Burgers.** 1989. Structure and function of the *Saccharomyces cerevisiae CDC2* gene encoding the large subunit of DNA polymerase III. EMBO J. **8:**1849–1854.
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. **Coen, D. M.** 1991. The implications of resistance to antiviral agents for herpesvirus drug targets and drug therapy. Antiviral Res. **15:**287–300.
7. **Davison, A. J., and J. E. Scott.** 1986. The complete DNA sequence of varicella-zoster virus. J. Gen. Virol. **67:**1759–1816.
8. **Earl, P. L., E. V. Jones, and B. Moss.** 1986. Homology between DNA polymerases of poxviruses, herpesviruses, and adenoviruses: nucleotide sequence of the vaccinia virus DNA polymerase gene. Proc. Natl. Acad. Sci. USA **83:**3659–3663.
9. **Garnier, J., D. J. Osguthorpe, and B. Robson.** 1978. Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:**97–120.
10. **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.
11. **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 analyses of the herpes simplex virus DNA polymerase gene predict a C-terminal substrate binding domain. Proc. Natl. Acad. Sci. USA **82:**7969–7973.
12. **Gingeras, T. R., D. Sciaky, R. E. Gelinas, J. Bing-Dong, C. E. Yen, M. M. Kelly, P. A. Bullock, B. L. Parsons, K. E. O'Neill, and R. J. Roberts.** 1982. Nucleotide sequences from the adenovirus-2 genome. J. Biol. Chem. **257:**13475–13491.
13. **Knopf, C. W., and K. Weisshart.** 1988. The herpes simplex virus DNA polymerase: analysis of the functional domains. Biochim. Biophys. Acta **951:**298–314.
14. **Ollis, D. L., P. Brick, R. Hamlin, N. G. Xuong, and T. A. Steitz.** 1985. Structure of large fragment of *Escherichia coli* DNA polymerase I complexed with dTMP. Nature (London) **313:**762–766.
15. **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.
16. **Quinn, J. P., and D. J. McGeoch.** 1985. DNA sequence of the region in the genome of herpes simplex virus type 1 containing the genes for DNA polymerase and the major DNA binding protein. Nucleic Acids Res. **13:**8143–8163.
17. **Sacks, S. L., R. J. Wanklin, D. E. Reece, K. A. Hicks, K. L. Tyler, and D. M. Coen.** 1989. Progressive esophagitis from acyclovir-resistant herpes simplex. Clinical roles for DNA polymerase mutants and viral heterogeneity? Ann. Intern. Med. **111:**893–899.
18. **Spicer, E. K., J. Rush, C. Fung, L. J. Reha-Krantz, J. D. Karam, and W. H. Konigsberg.** 1988. Primary structure of T4 DNA polymerase. Evolutionary relatedness to eucaryotic and other procaryotic DNA polymerases. J. Biol. Chem. **263:**7478–7486.
19. **Tomalski, M. D., J. Wu, and L. K. Miller.** 1988. The location, sequence, transcription, and regulation of a baculovirus DNA polymerase gene. Virology **167:**591–600.
20. **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 prokaryotic and eukaryotic replicative DNA polymerases. EMBO J. **7:**37–47.
21. **Yoshikawa, H., and J. Ito.** 1982. Nucleotide sequence of the major early region of bacteriophage φ 29. Gene **17:**323–335.