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Comparison of human cytomegalovirus DNA polymerase activity for ganciclovir-resistant and -sensitive clinical strains

Mieko Kariya, Seiichiro Mori, Yoshito Eizuru *

Division of Persistent and Oncogenic Viruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan

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Abstract

Previously, we found that a ganciclovir (GCV)-resistant clinical human cytomegalovirus (HCMV) isolate had an amino acid substitution at codon 501 (Leu → Phe) in the δ-region C of the DNA polymerase gene. DNA polymerases have now been (partially) purified from both the GCV-resistant and sensitive parental strains and the activity of DNA polymerase and 3′-5′ exonuclease compared. With respect to DNA polymerase activity, the Michaelis constant (K_m) and maximum velocity (V_{max}) of the GCV-resistant strain for the DNA template were lower than those of the GCV-sensitive strain. With respect to 3′-5′ exonuclease activity, the K_m and V_{max} of the GCV-resistant strain for the DNA substrate in the presence of ammonium sulfate were lower than those of the GCV-sensitive strain, while being similar in the absence of ammonium sulfate. Although the polymerase activity of the two strains showed almost the same sensitivity for the different polymerase inhibitors, the 3′-5′ exonuclease activity of the GCV-resistant strain was more resistant to these inhibitors than that of the GCV-sensitive strain.

Keywords: Ganciclovir; Cidofovir; Resistant virus; DNA polymerase; 3′-5′exonuclease

1. Introduction

The human cytomegalovirus (HCMV) is a major opportunistic viral pathogen causing serious sight- and life-threatening diseases in immunocompromised hosts, such as recipients of organ transplants and patients with acquired immunodeficiency syndrome (AIDS) (Britt and Alford, 1996). Antiviral drugs currently available for HCMV disease management include ganciclovir (GCV), foscarnet (PFA), and cidofovir (CDV). GCV is most widely used in clinical practice, both for induction and maintenance therapy and for prophylaxis in groups at high risk for HCMV disease. PFA is usually used for rescue therapy in patients with no response to GCV (Jacobson et al., 1991). CDV is the most recently approved anti-HCMV drug for HCMV retinitis treatment in AIDS patients (Lalezari et al., 1997). Long-

* Corresponding author. Tel.: +81-99-2755935; fax: +81-99-2755937.

E-mail address: ccvdgan@med3.kufm.kagoshima-u.ac.jp (Y. Eizuru)

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term treatment with anti-HCMV drugs, often necessary to prevent HCMV disease in immunocompromised hosts, may allow drug-resistant mutants to appear.

Viral DNA polymerase is the ultimate target for the currently available anti-HCMV drugs. GCV, a nucleoside analog, is monophosphorylated by virus-encoded UL97 phosphotransferase (Littler et al., 1992; Sullivan et al., 1992) and is further di- and tri-phosphorylated by cellular enzymes (Baldanti et al., 1995). GCV-triphosphate is the active form that is the substrate and inhibitor for viral DNA polymerase. GCV-resistant mutants can thus result from mutation(s) in either the phosphotransferase gene (UL97) or DNA polymerase gene (UL54), or both. In fact, the majority of GCV-resistant clinical isolates have a point mutation or deletion in UL97, while some contain mutation(s) in UL54, or in both UL54 and UL97 (Wolf et al., 1995; Baldanti et al., 1996; Chou et al., 1997; Erice et al., 1997; Smith et al., 1997; Harada et al., 1997; Baldanti et al., 1998). GCV-resistant mutants possessing mutation(s) in the conserved region IV, δ-region C, and V of UL54 exhibit cross-resistance to CDV but not to PFA (Erice et al., 1997; Smith et al., 1997; Harada et al., 1997; Cihlar et al., 1998a; Cihlar et al. 1998b).

We have isolated a clinical isolate, resistant to both GCV and CDV, from a patient with HCMV retinitis-encephalitis (Sasaki et al., 1997). Molecular analysis showed that the mutant had a point mutation at codon 501 in the δ-region C of UL54, which may lead to an alteration of both DNA polymerase and 3′-5′ exonuclease activities (Harada et al., 1997). The enzymatic activity of UL97 phosphotransferase has been studied and compared for wild type and mutants with UL97 mutation (Lurain et al., 1994). The enzymatic (DNA polymerase and 3′-5′ exonuclease) activity of UL54 mutants has not been completely clarified. Only Cihlar et al. (1998b) reported one mutant in which 3′-5′ exonuclease activity was severely compromised. We partially purified DNA polymerases from GCV-resistant and GCV-sensitive parental strains and compared their DNA polymerase and 3′-5′ exonuclease activities.

2. Materials and methods

2.1. Cell and viruses

Human embryonic lung fibroblasts (HEL) were grown in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, kanamycin (60 μg/ml), and 0.12% $NaHCO_3$. Both GCV-resistant and sensitive viruses were isolated from a patient with HCMV retinitis-encephalitis (Sasaki et al., 1997). They were plaque-purified twice and designated 93-lR and 9l-7S (Harada et al., 1997).

2.2. Chemicals

MEM was purchased from Nissui Pharmaceutical Co., Ltd., Tokyo, and fetal bovine serum from Flow Laboratories Inc., Irvine, VA. Bovine serum albumin (BSA) and DNase were purchased from Sigma, St. Louis, MO. The nucleosides (dNTPs, ddTTP, ddGTP and GMP), PAA (phosphonoacetic acid) and aphidicolin were obtained from Sigma. DEAE-cellulose, phosphocellulose, and glass filter paper were obtained from Whatman, London. [^{3}H]-dTTP and [^{3}H]-dGTP were obtained from Moravek. Ultrafiltratation membrane, Ultrafree CL, was obtained from Millipore, MA.

2.3. Partial purification of DNA polymerase

DNA polymerases were partially purified from infected cells according to the methods described elsewhere by Ochiai et al. with slight modification (1992). Confluent monolayers in culture bottles were infected with either GCV-resistant 93-1 R or sensitive 91-7S at a multiplicity of infection (MOI) of 1.0. Infected cells were harvested on day 5 after infection and washed once in phosphate-buffered saline (pH 7.4). After suspension in extraction buffer consisting of 20 mM Tris–HCl (pH 7.5) and 1 mM dithiothreitol (DTT), they were disrupted by ultrasonication. The crude extract was mixed with an equal volume of 3.4 M NaCl, 10 mM EDTA, and 1 mg/ml of BSA, and left on ice for 40 min. The mixture was centrifuged at 37 000 × g for 20 min at 4°C. The supernatant

containing nucleic lysate was dialyzed overnight against 50 mM Tris–HCl (pH 7.5), 0.5 mM DTT, 0.2% Nonidet P-40, and 20% glycerin (buffer A). After centrifugation at 24 000 $\times g$ for 20 min, the supernatant was batch-absorbed in DEAE cellulose preequilibrated with buffer A for 3 h. The mixture was packed into a column (1.0 × 10 cm) and eluted with a 50 ml gradient of 0.0–0.3 M KCl in buffer A. An equal aliquot from each fraction was assayed for DNA polymerase activity in the presence of 150 mM $(NH_4)_2SO_4$. Fractions eluted at 0.20–0.28 M KCl were pooled and dialyzed overnight against 0.2 M KCl, 50 mM Tris–HCl (pH 6.8), 0.5 mM DTT, and 20% glycerin (buffer B). The dialysate was batch-absorbed in phosphocellulose preequilibrated with buffer B containing 500 μg/ml of BSA. The mixture was packed into a column (1.0 × 10 cm) and eluted with a 50 ml gradient of 0.20–0.45 M KCl in buffer B.

Fractions eluted at 0.25–0.36 M KCl were pooled and supplemented with 500 μg/m1 of BSA, then dialyzed overnight against 50 mM Tris–HCl (pH 6.8), 0.5 mM DTT, and 20% glycerin to remove salt. Protein content exceeding 100 kDa of the final sample was measured by the Lowry method after washing and changing the buffer solution on a 100 kDa ultrafiltration membrane and removing BSA and other materials under 100 kDa. Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis was applied for sample purity.

2.4. Assay of DNA polymerase activity

DNA polymerase activity was assayed by the method of Mar et al. (1985) with slight modification. Native salmon sperm DNA in 10 mM Tris–HCl (pH 7.4) and 5 mM $MgCl_2$ was activated by DNase (0.1 unit/mg DNA) at 37°C for 25 min and heated at 60°C for 10 min. The standard reaction mixture consisted of 20 mM Tris–Cl (pH 8.0), 10 mM $MgCl_2$, 0.4 mM DTT, and 1 mg/ml of BSA (polymerase buffer); 0.2 mg/ml of activated salmon sperm DNA; 100 μM dNTP and l μM [^{3}H]-dTTP (2.22-3.33 TBq/mmol); and 10 μl of enzyme solution (9.12 μg protein/ml) in a total volume of 50 μl. The mixture was allowed to react at 37°C and the reaction was stopped at appropriate times by adding 50 μl of 0.1 M EDTA and 2 mg/ml of BSA. After 100 μl of 10% trichloroacetic acid (TCA) was added to the reaction mixture, the acid-insoluble precipitate was collected on a glass filter (GF/A) and washed with cold 5% TCA and 100% ethanol. After drying, filters were soaked in scintillation cocktail and radioactivity was counted in a liquid scintillation counter (LSC-3500, Aloka Co., Ltd., Tokyo).

2.5. Assay of 3′-5′exonuclease activity

The reaction mixture (50 μl) consisted of polymerase buffer, 10 μg of [^{3}H]-labeled salmon sperm DNA, and a DNA polymerase sample. Salmon sperm DNA was labeled with [^{3}H]-dGTP and [^{3}H]-dTTP according to the method of Kühn and Knopf (1996). The reaction mixture was allowed to react at 37°C for appropriate times, and the reaction was stopped as described for the DNA polymerase assay. Acid-insoluble radioactivity was counted in a liquid scintillation counter.

3. Results

3.1. Susceptibility of GCV-sensitive and resistant viruses to polymerase inhibitors

We previously reported that the IC_{50} of PAA for the GCV-sensitive clinical isolate (91-7S) and GCV-resistant clinical isolate (93-1R) in the plaque-reduction assay was about 27 μM (Harada et al., 1997). The IC_{50} of aphidicolin for the GCV-sensitive strain was 0.44 μM, and for the resistant strain 0.32 μM. Thus, both strains showed a similar susceptibility towards PAA as well as aphidicolin.

3.2. Partial purification of HCMV DNA polymerase

To compare the activity of DNA polymerase and 3′-5′ exonuclease between GCV-sensitive and resistant strains, viral DNA polymerases were partially purified at 4°C from either GCV-sensitive or GCV-resistant HCMV-infected cells with a

combination of DEAE-cellulose (Fig. 1A) and phosphocellulose (Fig. 1B) column chromatography according to Ochiai et al. (1992) with modifications. The fractions, which contained DNA polymerase activity in the presence of 150 mM ammonium sulfate, were pooled. After desalting the dialysate, aliquots were stored at −80°C until further study. To confirm purity, we analyzed both the original assay sample and the 30-times concentrated sample by SDS-PAGE after washing on a 100 kDa ultrafiltration membrane. We saw only a single 140 kDa band in both lanes of

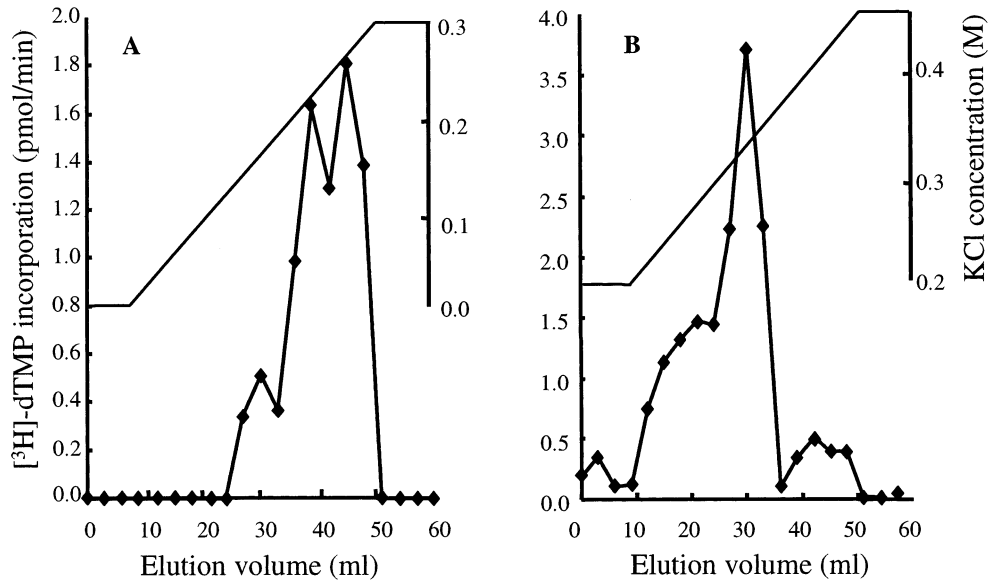

Fig. 1. Purification of HCMV DNA polymerase from GCV-resistant strain by (A) first DEAE-cellulose column and (B) second phosphocellulose column chromatography. Chromatography conditions are described in Section 2.

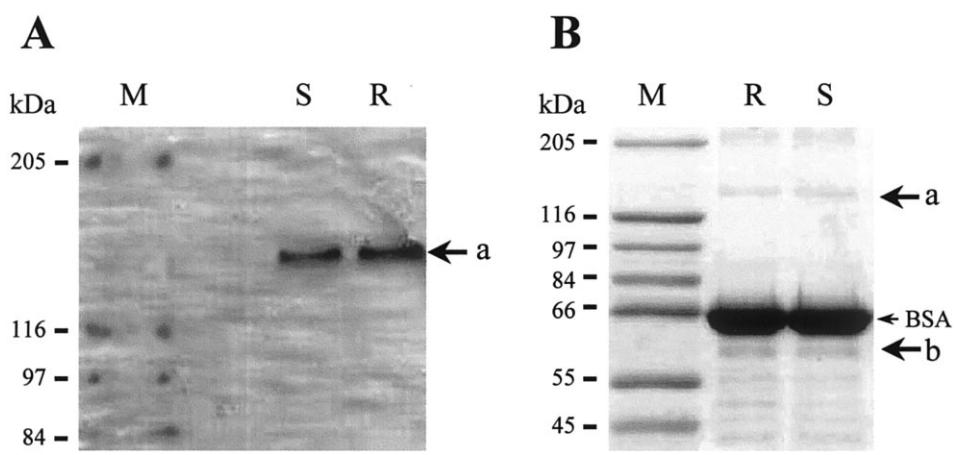

Fig. 2. PAGE of the sample (A) after cutting off the 'under 100 kDa' materials, and (B) the original assay sample. Concentrations of polyacrylamide gel were (A) 7% and (B) 9%. After separation, proteins were stained with Coomassie brilliant blue. a, 140 kDa DNA polymerase; b, 58 kDa processivity factor; M, molecular marker; S, GCV-sensitive; R, resistant sample.

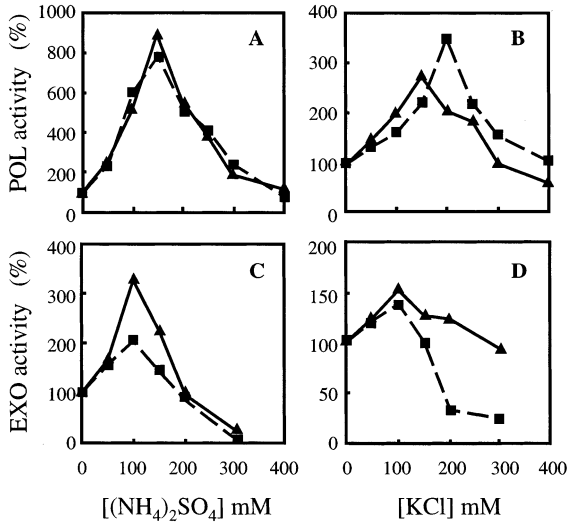

Fig. 3. Effects of ammonium sulfate (A, C) and KCl (B, D) on the activity of GCV-resistant (—) and -sensitive (----) HCMV DNA polymerase. Conditions are described in Section 2. Activity is expressed as the percentage of activity in the absence of $(NH_4)_2SO_4$ or KCl.

Table 1
Functional comparison of GCV-resistant and -sensitive HCMV DNA polymerase

Enzyme	Specific activity (units[a])	
	POL activity (%)	EXO activity (%)
Resistant	0.66 ± 0.06 (116)	1.34 ± 0.08 (152)
Sensitive	0.57 ± 0.06 (100)	0.88 ± 0.07 (100)

[a] One unit of enzyme activity corresponds to the incorporation of 1 μmol of dTTP into the template DNA (POL activity) and with the release of 1 μmol of dTMP or dGMP from substrate DNA (EXO activity) per min, per mg protein, in the presence of 150 mM $(NH_4)_2SO_4$ as described in Section 2. Mean of three experimental data $\pm$ SE.

GCV-sensitive and -resistant samples (Fig. 2A): 58 kDa processivity factor was also indicated in original assay samples (Fig. 2B), although many other bands were shown. Samples cut off under 100 kDa were measured for protein content by the Lowry method. The GCV-resistant sample contained 15.04 μg/ml protein and the GCV-sensitive sample 9.12 μg/ml.

3.3. DNA polymerase activity of GCV-resistant and -sensitive strains

Although the effect of salts and pH on HCMV DNA polymerase activity was studied by Nishiyama et al. (1983), we are the first to study whether differences existed in optimal conditions between GCV-sensitive and -resistant strains. DNA polymerase activity was stimulated by 150 mM $(NH_4)_2SO_4$ 7.9-fold for the GCV-sensitive strain and 9.2-fold for the GCV-resistant strain (Fig. 3A). The optimal KCl concentration differed slightly: 150 mM for the GCV-resistant and 200 mM for the GCV-sensitive DNA polymerases (Fig. 3B). The optimal pH was 8.0 for both DNA polymerases (data not shown). Further experiments were conducted in the presence of 150 mM $(NH_4)_2SO_4$ at pH 8.0. This concentration of ammonium sulfate inhibited contaminants of host cell DNA polymerase activity (Nishiyama et al., 1983). Specific DNA polymerase activity was almost identical for both strains (Table 1).

Michaelis constants (K_m) and maximum velocities (V_{max}) for the template DNA and substrate were determined by Lineweaver–Burk plots. V_{max} and K_m for template DNA were lower for the GCV-resistant strain than for the GCV-sensitive strain (Table 2). In contrast, K_m and V_{max} values for dTTP and dGTP were almost the same for the two strains (Table 2). DNA polymerase activity of both strains was inhibited to the same degree by the noncompetitive inhibitors phosphonoacetic acid (PAA) and aphidicolin (data not shown).

Although the competitive inhibitor ddTTP showed almost no effect on DNA polymerase activity of the two strains, another competitive inhibitor, ddGTP, inhibited slightly DNA polymerase activity of the GCV-resistant strain but not that of the GCV-sensitive strain (Fig. 4A and B).

3.4. Comparison of 3′-5′ exonuclease activity between GCV-resistant and sensitive strains

The optimal concentration of $(NH_4)_2SO_4$ and KCl for 3′-5′ exonuclease activity of both strains was 100 mM (Fig. 3C and D). Under these conditions, the 3′-5′ exonuclease activity of the GCV-re-

Table 2
Comparison of V_{max} and K_m of the GCV-resistant and -sensitive HCMV DNA polymerase

$(NH_4)_2SO_4$	POL			EXO	
	DNA+	dTTP+	dGTP+	DNA−	DNA+
V_{max} (units[a])					
Resistant	1.16	2.22	2.54	3.47	4.87
Sensitive	4.07	2.33	2.65	2.95	8.43
Resistant/sensitive	0.286	0.953	0.992	1.18	0.578
K_m	(μg/ml)	(μM)	μM)	(μg/ml)	(μg/ml)
Resistant	341	0.60	0.91	472	234
Sensitive	1990	0.62	0.70	463	1091
Resistant/sensitive	0.171	0.965	1.30	1.02	0.214

[a] One unit of enzyme activity corresponds to the incorporation of 1 μmol of dTTP into the template DNA (POL activity) and with the release of 1 μmol of dTMP and dGMP from substrate DNA (EXO activity), per min, per mg protein.

sistant strain was higher than that of the GCV-sensitive strain (Table 1). V_{max} and K_m for the substrate DNA were determined by Lineweaver–Burk plots in the presence or absence of $(NH_4)_2SO_4$. Both V_{max} and K_m were lower in the GCV-resistant strain than in the GCV-sensitive strain only in the presence of ammonium sulfate (Table 2). The 3′-5′ exonuclease activity of the GCV-resistant virus was more resistant to inhibition by both non/uncompetitive and competitive inhibitors than that of the GCV-sensitive virus (Fig. 4C and Fig. 5).

4. Discussion

In a previous study, Harada et al. (1997) reported the isolation of a GCV-resistant mutant having a point mutation at codon 501 in the δ-region C of the UL54 gene (Fig. 6). Because this mutation occurs in the conserved region of the polymerase, close to the conserved region of Exo III of 3′-5′ exonuclease, we expected that both activities would be affected by the mutation. We partially purified the polymerases from virus-infected cells and compared the activity of the DNA polymerase and 3′-5′ exonuclease of the resistant mutant and sensitive parental strains.

In polymerase activity, the resistant strain showed almost the same specific activity compared to that of the parental sensitive strain, but showed a slight difference in susceptibility to ddGTP, but not ddTTP, in inhibition experiments. CDV-diphosphate and GCV-triphosphate could not be included in inhibition experiments because these compounds were not available. Our results are comparable to those of Cihlar et al. (1998a), where the mutant at codon 513 of the

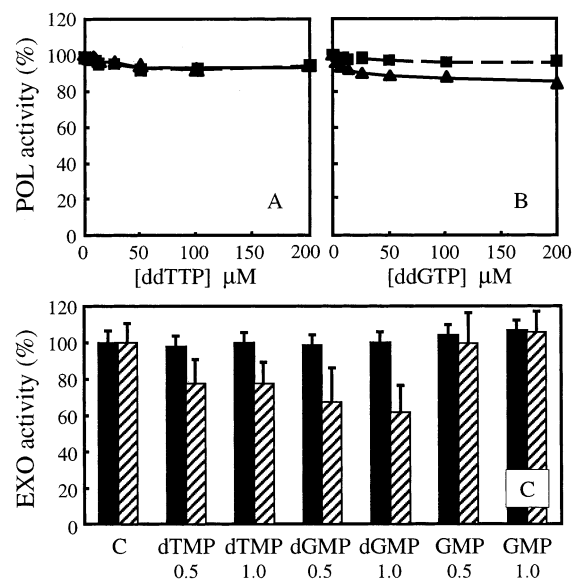

Fig. 4. Susceptibility of HCMV DNA polymerase from GCV-resistant strain (A, B, —; C, ■) and sensitive strain (A, B,-----; C, ▨) to competitive inhibitors. A, B, polymerase activity; C, 3′-5′ exonuclease activity; C, controls; 0.5 and 1.0 are in μM.

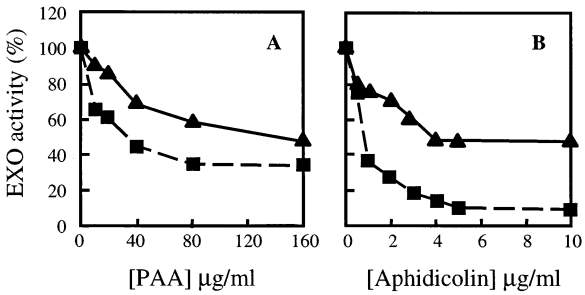

Fig. 5. Susceptibility of HCMV 3′-5′ exonuclease activity from GCV-resistant strain (—) and -sensitive strain (-----) to non/uncompetitive inhibitors. A, phosphonoacetic acid; B, aphidicolin.

δ-region C showed almost the same specific polymerase activity as that of the parental strain. Although no significant difference was seen between the codon 513 mutant and parental strain enzyme with regard to their sensitivity to CDV-diphosphate and GCV-triphosphate, the mutant decreased the susceptibility to CDV and GCV of the *ori* Lyt plasmid replication in transient transfection/infection assay (Cihlar et al., 1998b). Unfortunately, the sophisticated transient transfection/infection assay system was not available to us.

In 3′-5′ exonuclease activity, the codon 501 mutant showed higher specific activity than the parental sensitive strain. This contrasted sharply to the codon 513 mutant reported by Cihlar et al. (1998b), which was virtually deficient in 3′-5′ exonuclease function. Based on homology with α-DNA polymerases, it has been proposed that four Exo motifs — Exo I, I′, II, III — in the N-terminal portion of the HCMV DNA polymerase constitute the 3′-5′ exonuclease site (Bernad et al., 1989; Blanoco et al., 1991). Specific mutations within the Exo motifs of herpes simplex virus type 1 (HSV-1) DNA polymerase impair the 3′-5′ exonuclease function (Kühn and Knopf, 1996; Hwang et al., 1997). Both amino acids at residues 501 and 513 are conserved among DNA polymerases in δ-region C, but residue 513 is closer to the Exo III domain. This is why the 513 mutation almost abolished 3′-5′ exonuclease activity. In contrast, the 501 mutation enhanced 3′-5′ exonuclease activity, and decreased its susceptibility to competitive and non/uncompetitive inhibitors. A comparison of the mutational frequency of the K513N mutant virus (exonuclease activity compromised) and L501F mutant virus (exonuclease activity enhanced) may help to clarify if primary polymerase resistance mutations affecting exonuclease activity could in fact influence the frequency of selection of the secondary resistance mutations.

References

Baldanti, F., Silini, E., Sarasini, A., Talarico, C.L., Stanat, S.C., Biron, K.K., Furione, M., Bono, F., Palù, G., Gerna, G., 1995. A three-nucleotide deletion in the UL97 open reading frame is responsible for the ganciclovir resistance of a human cytomegalovirus clinical isolate. J. Virol. 69, 796–800.

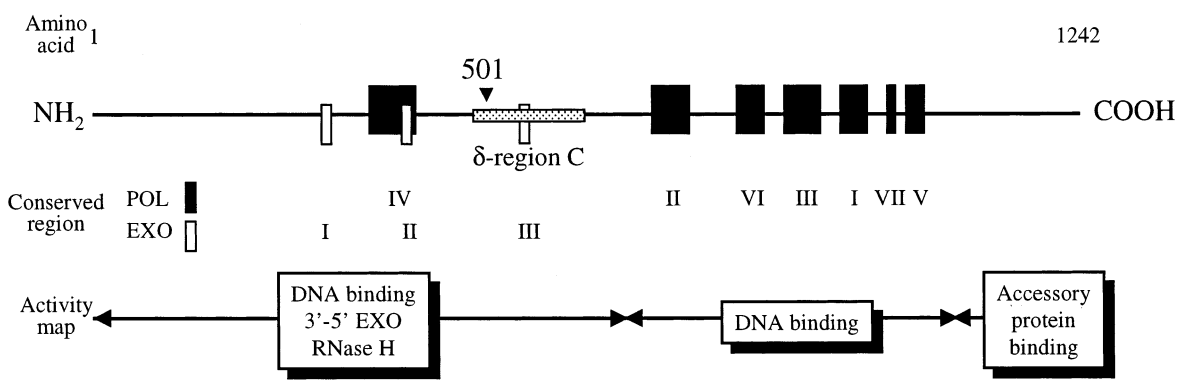

Fig. 6. HCMV DNA polymerase polypeptide.

Baldanti, F., Underwood, M.R., Stanat, S.C., Biron, K.K., Chou, S., Sarasini, A., Silini, E., Gerna, G., 1996. Single amino acid changes in the DNA polymerase confer foscarnet resistance and slow-growth phenotype, while mutations in the UL97-encoded phosphotransferase confer ganciclovir resistance in three double-resistant human cytomegalovirus strains recovered from patients with AIDS. J. Virol. 70, 1390–1395.

Baldanti, F., Underwood, M.R., Talarico, C.L., Simoncini, L., Sarasini, A., Biron, K.K., Gerna, G., 1998. The Cys607 → Tyr change in the UL97 phosphotransferase confers ganciclovir resistance to two human cytomegalovirus strains recovered from two immunocompromised patients. Antimicrob. Agents Chemother. 42, 444–446.

Bernad, A., Blanco, L., Lazaro, J.M., Martin, G., Salas, M., 1989. A conserved 3′-5′exonuclease active site in prokaryotic and eukaryotic DNA polymerases. Cell 59, 219–228.

Blanco, L., Bernad, A., Blanco, M.A., Salas, M., 1991. A general structure for DNA-dependent DNA polymerases. Gene 100, 27–38.

Britt, W.J., Alford, C.A., 1996. Cytomegalovirus. In: Fields, B.N., Knipe, D.M., Howly, P.M. (Eds.), Virology, 3rd ed. Raven Press, New York, pp. 2493–2524.

Chou, S., Marousek, G., Guentzel, S., Follansbee, S.E., Poscher, M.E., Lalezari, J.P., Miner, R.C., Drew, W.L., 1997. Evolution of mutations conferring multidrug resistance during prophylaxis and therapy for cytomegalovirus disease. J. Infect. Dis. 176, 786–789.

Cihlar, T., Fuller, M.D., Cherrington, J.M., 1998a. Characterization of drug resistance-associated mutations in the human cytomegalovirus DNA polymerase gene by using recombinant mutant viruses generated from overlapping DNA fragments. J. Virol. 72, 5927–5936.

Cihlar, T., Fuller, M.D., Mulato, A.S., Cherrington, J.M., 1998b. A point mutation in the human cytomegalovirus DNA polymerase gene selected in vitro by cidofovir confers a slow replication phenotype in cell culture. Virology 248, 382–393.

Erice, A., Gil-Roda, C., Pérez, J.-L., Balfour, H.H.Jr., Sannerud, K.J., Hanson, M.N., Boivin, G., Chou, S., 1997. Antiviral susceptibilities and analysis of UL97 and DNA polymerase sequences of clinical cytomegalovirus isolates from immunocompromised patients. J. Infect. Dis. 175, 1087–1092.

Harada, K., Eizuru, Y., Isashiki, Y., Ihara, S., Minamishima, Y., 1997. Genetic analysis of a clinical isolate of human cytomegalovirus exhibiting resistance against both ganciclovir and cidofovir. Arch. Virol. 142, 215–225.

Hwang, Y.T., Liu, S.-Y., Coen, D.M., Hwang, C.B.C., 1997. Effects of mutations in the Exo III motif of the herpes simplex virus DNA polymerase gene on enzyme activities, viral replication. and replication fidelity. J. Virol. 71, 779l–7798.

Jacobson, M.A., Drew, W.L., Feinberg, J., O'Donnell, J.J., Whitmore, P.V., Miner, R.D., Parenti, D., 1991. Foscarnet therapy for ganciclovir-resistant cytomegalovirus retinitis in patients with AIDS. J. Infect. Dis. 163, 1348–1351.

Kühn, F., Knopf, C., 1996. Herpes simplex virus type 1 DNA polymerase: mutational analysis of the 3′-5′-exonuclease domain. J. Biol. Chem. 271 (46), 29245–29254.

Lalezari, J.P., Stagg, R.J., Baruch, D., 1997. Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. Ann. Intern. Med. 126, 257–263.

Littler, E., Stuart, A.D., Chee, M.S., 1992. Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. Nature 358, 160–162.

Lurain, N.S., Spafford, L.E., Thompson, K.D., 1994. Mutation in the UL97 open reading frame of human cytomegalovirus strains resistant to ganciclovir. J. Virol. 68, 4427–4431.

Mar, E.-C., Chiou, J.-F., Cheng, Y.-C., Huang, E.-S., 1985. Human cytomegalovirus-induced DNA polymerase and its interaction with the triphosphates of 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-methyluracil, -5-iodocytosine, and -5-methylcytosine. J. Virol. 56, 846–851.

Nishiyama, Y., Maeno, K., Yoshida, S., 1983. Characterization of human cytomegalovirus-induced DNA polymerase and the associated 3′-to-5′, exonuclease. Virology 124, 221–231.

Ochiai, H., Kumura, K., Minamishima, Y., 1992. Murine cytomegalovirus DNA polymerase: purification, characterization and role in the antiviral activity of acyclovir. Antiviral Res. 17, 1–16.

Sasaki, T., Okayama, A., Eizuru, Y., Murai, K., Matsuoka, H., Uno, H., Minamishima, Y., Kataoka, H., Koono, M., Matsuura, Y., Naoi, N., Sawada, A., Tachibana, N., Tsubouchi, H., 1997. Progressive retinitis-encephalitis due to ganciclovir-resistant cytomegalovirus associated with aplastic anemia. Intern. Med. 36, 375–379.

Smith, I.L., Cherrington, J.M., Jiles, R.E., Fuller, M.D., Freeman, W.R., Spector, S.A., 1997. High-level resistance of cytomegalovirus to ganciclovir is associated with alterations in both the UL97 and DNA polymerase genes. J. Infect. Dis. 176, 69–77.

Sullivan, V., Talarico, C.L., Stanat, S.C., Davis, M., Coen, D.M., Biron, K.K., 1992. A protein kinase homologue controls phosphorylation of ganciclovir in human cytomegalovirus-infected cells. Nature 358, 162–164.

Wolf, D.G., Smith, I.L., Lee, D.J., Freeman, W.R., Flores-Aguilar, M., Spector, S.A., 1995. Mutations in human cytomegalovirus UL97 gene confer clinical resistance to ganciclovir and can be detected directly in patient plasma. J. Clin. Invest. 95, 257–263.