Antiviral Research 93 (2012) 199–203

Contents lists available at SciVerse ScienceDirect

Antiviral Research

journal homepage: www.elsevier.com/locate/antiviral

Short Communication

Genotypic characterization of herpes simplex virus DNA polymerase UL42 processivity factor

Sonia Burrel [a,b,*], Zaïna Aït-Arkoub [b], Henri Agut [a,b], David Boutolleau [a,b]

[a] UPMC Univ Paris 06, ER1 DETIV, F-75013, Paris, France
[b] Service de Virologie, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, F-75013, Paris, France

ARTICLE INFO

Article history:
Received 26 July 2011
Revised 14 October 2011
Accepted 19 October 2011
Available online 28 October 2011

Keywords:
Herpes simplex virus
UL42 processivity factor
UL30 DNA polymerase
Natural polymorphism
Antiviral resistance

ABSTRACT

The herpes simplex virus (HSV) DNA polymerase is composed of the UL30 catalytic subunit and the UL42 processivity factor. The UL42 subunit increases the processivity of the polymerase along the DNA template during replication. The molecular mechanisms of HSV resistance to drugs interfering with viral DNA synthesis reported so far mainly rely on modifications of the viral thymidine kinase and DNA polymerase. We aimed to extensively describe the genetic variations of HSV UL42 processivity factor and to evaluate its potential involvement in resistance to antivirals. The full-length UL42 gene sequence of HSV was investigated among two laboratory strains (KOS and gHSV-2), 94 drug-sensitive clinical isolates and 25 phenotypically ACV-resistant clinical isolates. This work provided extensive data about natural variability of UL42 processivity factor among both HSV-1 and HSV-2 strains and showed that this viral protein is highly conserved among HSV strains, with a weaker variability for HSV-2. The analysis of 25 HSV clinical isolates exhibiting ACV-resistance documented most of the previously reported mutations related to UL42 natural polymorphism in addition to some unpreviously described polymorphisms. Surprisingly, a single-base deletion in UL42 gene sequence leading to a frameshift in the C-terminal region was identified among 3 HSV clinical isolates. From this preliminary study, UL42 processivity factor did not seem to be likely involved in HSV resistance to antivirals.

Herpes simplex virus (HSV)-1 and HSV-2 might be associated with severe morbidity and mortality in immunocompromised individuals, in particular transplant recipients and AIDS patients (Dignani et al., 2002; Englund et al., 1990; Hill et al., 1991). The drugs currently used for the treatment of HSV infections, acyclovir (ACV) and its prodrug valacyclovir, foscarnet (FOS) and cidofovir (CDV), have provided a major advance in the management of HSV diseases (Gilbert et al., 2002). However, the antiviral effectiveness may be restricted due to significant toxicity or the emergence of viral drug-resistance. HSV resistance to ACV and FOS has been associated with modifications located in the UL23 thymidine kinase (TK) and UL30 DNA polymerase. ACV-resistance mainly stems from TK alterations due to the occurrence of either a shift of the translational reading frame or non-synonymous mutations. Resistance to FOS, and to a lesser extent to ACV, is related to amino acid changes within catalytic or conserved regions of the DNA polymerase (Burrel et al., 2010; Gilbert et al., 2002). As for other herpesviruses, HSV DNA polymerase complex consists of a heterodimer composed of a catalytic subunit (UL30) and an accessory protein (UL42) which increases the processivity of the enzyme (Loregian et al., 2000). UL42 processivity factor, consisting of a 488 and 470-amino-acid protein for HSV-1 and HSV-2, respectively, tethers DNA tightly as a monomer allowing prolonged association, thereby conferring extended DNA strand synthesis. Crystal structure data evidenced that the UL30/UL42 protein–protein interaction involves several specific residues located within the UL30 extreme C-terminal domain (codons 1200–1235) and the UL42 connector loop (codons 160–175) (Zuccola et al., 2000). The disruption of this interaction has been proposed as a potential target to hinder viral replication using oligopeptides corresponding to the very C-terminal region of the UL30 or peptidomimetic compounds (Loregian and Palu, 2005). Hitherto, only the identification of the UL30 and UL42 regions responsible for the physical and functional interaction of these two proteins has been the subject of extensive investigations. The aims of this work were to describe precisely the entire natural polymorphism of HSV UL42 processivity factor and to perform its genotypic characterization among drug-sensitive and drug-resistant HSV clinical isolates in order to evaluate its potential involvement in resistance to antivirals.

* Corresponding author. Address: Service de Virologie, Groupe Hospitalier Pitié-Salpêtrière, 83 boulevard de l'hôpital, F-75651 Paris Cedex 13, France. Tel.: +33 1 42 17 74 02; fax: +33 1 42 17 74 11.
E-mail address: sonia.burrel@psl.aphp.fr (S. Burrel).

0166-3542/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.antiviral.2011.10.016

Two laboratory strains (KOS and gHSV-2), 94 drug-sensitive (43 HSV-1 and 51 HSV-2) and 25 phenotypically drug-resistant clinical isolates were studied. The genotypic data concerning UL23 and UL30 genes have been previously described (Burrel et al., 2010). Viral DNA was extracted from viral stocks using QIAamp® DNA blood mini kit (Qiagen, Courtaboeuf, France). The full-length UL42 gene from HSV-1 (1467 base pairs [bp]) and HSV-2 (1413 bp) was amplified by nested PCR using the proofreading enzyme Expand High Fidelity (Roche, Meylan, France). Amplified products were sequenced using overlapping primer pairs with the Prism Big Dye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Courtaboeuf, France) and analyzed with the automated sequencer ABI 3730 Genetic Analyzer (Applied Biosystems). Primers used for gene amplification and sequencing are listed in Table 1. All nucleotide and amino acid sequencing results were compared with published sequences of the reference strains 17 (HSV-1) and HG52 (HSV-2) (GenBank accession numbers X14112 and Z86099, respectively) using Seqscape v2.5 software (McGeoch et al., 1985, 1987). All UL42 sequences determined in this study have been deposited in the GenBank database under accession numbers JF810705 through JF810823.

Among HSV drug-sensitive strains (2 laboratory strains and 94 clinical isolates), the interstrain identity at the nucleotide level of UL42 gene ranged from 98.8% to 99.8% and from 99.3% to 99.9% for HSV-1 and HSV-2, respectively. In comparison with reference sequences, 58 and 22 nucleotide substitutions were identified within the coding sequence for HSV-1 and HSV-2, respectively, corresponding to an average number of 9.1 and 4.4 per strain. Almost half of them (35/80) were synonymous polymorphisms. At the amino acid level, the interstrain identity of UL42 processivity factor was >98.4%, with the identification of 28 and 12 amino acid changes for HSV-1 and HSV-2, respectively, corresponding to 5.5% and 2.6% of the total codons of the protein. The number of amino acid changes per strain ranged from 1 to 7 and from 0 to 4 for HSV-1 and HSV-2, respectively. As presented in Table 2, the most frequent amino acid changes observed were P13H, G284A, L377S, H378Y and R404H for HSV-1, S328T, S351F and P389A for HSV-2. Surprisingly, one HSV-1 isolate harbored a guanosine deletion (Del 1G 1433) leading to the creation of a frameshift and the alteration of the extreme C-terminal amino acid sequence from the residue 479. All these changes were located outside conserved domains of the viral protein, except R404H and K411R (HSV-1), P389A and A399T (HSV-2), which were located within the conserved nuclear localization signal (NLS) of UL42 processivity factor (Fig. 1). Among the 25 drug-resistant HSV strains, the amino acid sequences were identical to those of reference strains for 3 isolates. For the other isolates, 15 out of the 40 amino acid changes previously evidenced in HSV drug-sensitive isolates were found. Moreover, new amino acid changes were identified: S21P, K105T, A353S, A419T, S434P and R397C for HSV-1, G93E and F457S for HSV-2 (Table 2). These changes were located outside the conserved/functional domains of the protein, except K105T and R397C (HSV-1) settled in the back face interacting with DNA and in the NLS, respectively (Figs. 1 and 2). One isolate harbored the association of 3 amino acid changes unpreviously described (A353S + A419T + S434P) (Table 2). For one HSV-1 resistant isolate, the frameshift deletion previously described Del 1G 1433 was found. One HSV-2 isolate also harbored a frameshift deletion (Del 1C 1185) affecting NLS motif from residue 389 (Fig. 1). Regarding HSV-1, one isolate exhibited the unpreviously described R1229I change located in C-terminal domain of UL30 DNA polymerase (data not shown) (Burrel et al., 2010). The intermolecular hydrogen bond involving this residue and Q171 residue in UL42 processivity factor is fundamental for the UL30/UL42 interaction (Fig. 2) (Loregian and Palu, 2005; Zuccola et al., 2000). No specific association of mutation within UL42 processivity factor and drug-resistance mutations within TK and/or DNA polymerase could be evidenced (data not shown). However, the use of a proofreading high fidelity enzyme for UL42 gene amplification cannot definitively rule out the possibility that some of the mutations described above might be the consequence of PCR artefacts.

This study aimed to analyze the natural variation of HSV UL42 processivity factor and its potential involvement in HSV resistance to antivirals. This work provides extensive data about UL42 natural polymorphism of both HSV-1 and HSV-2. Our results evidenced that UL42 processivity factor is highly conserved among 96 HSV drug-sensitive strains (2 laboratory strains and 94 clinical isolates), with an interstrain identity >98.4% at both nucleotide and amino acid levels, and a weaker variability for HSV-2. Similar results were previously reported for the UL30 catalytic subunit of HSV DNA polymerase (Burrel et al., 2010; Chiba et al., 1998). Given their essential role in replication, it appears logical that both UL42 and UL30 proteins are extremely conserved. Moreover, it has been reported that DNA polymerase processivity factors from human cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) (encoded by UL44 and U27 genes, respectively) also exhibited a low natural polymorphism (Boutolleau et al., 2009; Bonnafous et al., 2010). Regarding the 25 drug-resistant HSV isolates, 15 natural polymorphisms previously identified in the first part of our study were found (Table 2). However, 9 unpreviously described changes

Table 1
Primers used for amplification and sequencing of UL42 gene among HSV-1 and HSV-2 strains.

HSV type	Function	Name	Sequence (5′→3′)
HSV-1	First-round PCR (outer primers)	42H1-F1	R: CCTGGCTGCCATCAAAAC
		42H1-R1	F: GGCACACACATGAACCACAC
	Second-round PCR (inner primers)	42H1-F2	F: AAGCCCACCTCGTCACTGT
		42H1-R2	R: GACACGCGGGAAAGTGTT
	Sequence reaction	42H1-A	R: CGTTAAGGACCTTGGTGAGC
		42H1-B	F: CAGCGAGACGCTGATGAAG
		42H1-C	R: GGAGTCCTGGCTGTCTGTTG
		42H1-D	F: TGAAACCCCAGAAGATTTGC
		+42H1-F2 and 42H1-R2	
HSV-2	First-round PCR (outer primers)	42H2-F1	F: CTCGGGTAGCCACTGCTCT
		42H2-R1	R: AACCACACACGACAGCCATA
	Second-round PCR (inner primers)	42H2-F2	F: CTCGTCTCTGCGGATTGTC
		42H2-R2	R: GGGGACACACTACCCCATTT
	Sequence reaction	42H2-A	R: CGTTCACCACCTTCGTGAG
		42H2-B	F: CGAGCTTCGCGGTACTACTC
		42H2-C	R: AGGAGGAGTCCTGGCTGTC
		42H2-D	F: AGCGGGTCTGCCTGAACT
		+42H2-F2 and 42H2-R2	

Table 2
Nucleotide and amino acid changes within HSV UL42 processivity factor among 96 drug-sensitive strains and 25 phenotypically ACV-resistant clinical isolates.

HSV-1			HSV-2		
Mutations	Drug-sensitive viruses[a] (n = 44)	Drug-resistant viruses[b] (n = 11)	Mutations	Drug-sensitive viruses[a] (n = 52)	Drug-resistant viruses[b] (n = 14)
P13H	23 (52.3)	4 (36.4)	P12T	1 (1.9)	0 (0)
V14M	1 (2.3)	1 (9.1)	**G93E + F457S**	0 (0)	1 (7.1)
S18P	2 (4.5)	0 (0)	R216H	1 (1.9)	0 (0)
S21P	0 (0)	1 (9.1)	A253T	1 (1.9)	0 (0)
I66L	1 (2.3)	0 (0)	S328T	30 (57.7)	7 (50)
R92S	2 (4.5)	1 (9.1)	S351F	24 (46.2)	6 (42.9)
K105T	0 (0)	1 (9.1)	R373W	1 (1.9)	0 (0)
R122W	1 (2.3)	0 (0)	P375T	1 (1.9)	0 (0)
V219I	1 (2.3)	0 (0)	P389A	43 (82.7)	10 (71.4)
G284A	26 (59.1)	5 (45.5)	A399T	1 (1.9)	0 (0)
V296T	1 (2.3)	0 (0)	R422G	3 (5.8)	1 (7.1)
S333P	1 (2.3)	0 (0)	G431D	1 (1.9)	0 (0)
A334P	1 (2.3)	0 (0)	A456T	1 (1.9)	0 (0)
S337L	1 (2.3)	0 (0)	**Del 1C 1185 (FS)**	0 (0)	1 (7.1)
A353S + A419T + S434P	0 (0)	1 (9.1)			
S358N	1 (2.3)	0 (0)			
D361E	1 (2.3)	1 (9.1)			
D366N	1 (2.3)	1 (9.1)			
A369V	1 (2.3)	0 (0)			
L377S	44 (100)	11 (100)			
H378Y	30 (68.2)	6 (54.5)			
R397C	0 (0)	1 (9.1)			
R404H	24 (54.5)	2 (18.2)			
K411R	1 (2.3)	0 (0)			
A423T	1 (2.3)	0 (0)			
D424N	1 (2.3)	0 (0)			
V426A	1 (2.3)	1 (9.1)			
R443S	1 (2.3)	0 (0)			
G452V	2 (4.5)	0 (0)			
G465R	1 (2.3)	0 (0)			
G478A	1 (2.3)	0 (0)			
Del 1G 1433 (FS)	1 (2.3)	1 (9.1)			

FS, frameshift; Del, deletion. Values are displayed as n (%) for HSV strains. Amino acid changes in bold were reported only in drug-resistant HSV clinical isolates.
[a] HSV strains (2 laboratory strains and 94 clinical isolates) either characterized as ACV- and FOS-sensitive according to PRA (n = 44) or recovered from patients who had not received any prior anti-HSV therapy (n = 52) (Burrel et al., 2010).
[b] HSV clinical isolates exhibiting only ACV-resistance (n = 24) or both ACV- and FOS-resistance (n = 1) (Burrel et al., 2010).

including S21P, K105T, A353S, A419T, S434P and R397C for HSV-1, G93E, F457S and Del 1C 1185 for HSV-2, have also been evidenced (Table 2). With regard to the assumed functional domains of UL42 processivity factor, 2 of them (i.e., K105T and R397C) were localized in crucial regions in HSV-1 isolates (Figs. 1 and 2). K105T change is located in UL42 basic face (side opposite to UL30 binding site). In this region, the substitution of any of the four conserved arginine residues (R113, R182, R279 and R280) with alanines results in decreased DNA binding affinity, but mutants retain affinity for UL30 C-terminal residues (Randell et al., 2005). This basic face also includes less-well conserved residues as lysine residues K105 and K275. The amino acid change K105T (basic to uncharged polar amino acid change) might be associated with the disruption of the physical interaction between UL42 and DNA (Komazin-Meredith et al., 2008). UL42 processivity factor is imported to the nucleus through a process that requires a C-terminally located NLS (Fig. 2). R397 is the last of the seven residues of the $PTTKRGR_{397}$ monopartite NLS motif. The substitution R to C is likely to interfere with nuclear localization. Nevertheless, it has been shown that mutant derivatives of UL42 accessory protein lacking NLS are partially relocalized into the cell nucleus upon coexpression with UL30 DNA polymerase (Alvisi et al., 2008). Surprisingly, a frameshift in UL42 gene due to the single-base deletion Del 1G 1433, inducing the disruption of the extreme UL42 C-terminal amino acid sequence from residue 479, was identified among one drug-sensitive and one drug-resistant HSV-1 isolates (Table 2). However, it has been previously demonstrated that the first 338 residues of UL42 are sufficient for viral replication (Gao et al., 1993). The exact implication of these different mutations located within the conserved domain of HSV UL42 processivity factor regarding viral fitness require further investigations using marker transfer experiments. From this preliminary study, no mutation in UL42 processivity factor was significantly associated with HSV resistance to currently used antivirals due to mutations located in UL23 TK and/or in UL30 DNA polymerase. New anti-HSV drugs that inhibit viral replication by disrupting the interaction between the 2 subunits of the virus DNA polymerase complex might constitute in the future an alternative therapeutic to the available molecules owning the limits of antiviral resistance and toxicity (Loregian and Palu, 2005). It is noteworthy that the UL30/UL42 interaction domains are highly conserved. Thus, in this work, R1229I change within UL30 catalytic subunit DNA polymerase was the unique change evidenced in these regions in an ACV-resistant HSV-1 clinical isolate (Burrel et al., 2010). The side-chain of R1229 residue is involved in a hydrogen-bonding link with UL42 residue Q171 located in the connector loop (Fig. 2). One can expect that resistance to these new molecules might be linked to mutations within UL42 protein. Therefore, genotypic tools concerning HSV UL42 processivity factor to improve the knowledge of natural polymorphism and to detect potential drug resistance mutations in a clinically relevant time frame will be very useful.

Disclosure statement

The authors declare no competing interests.

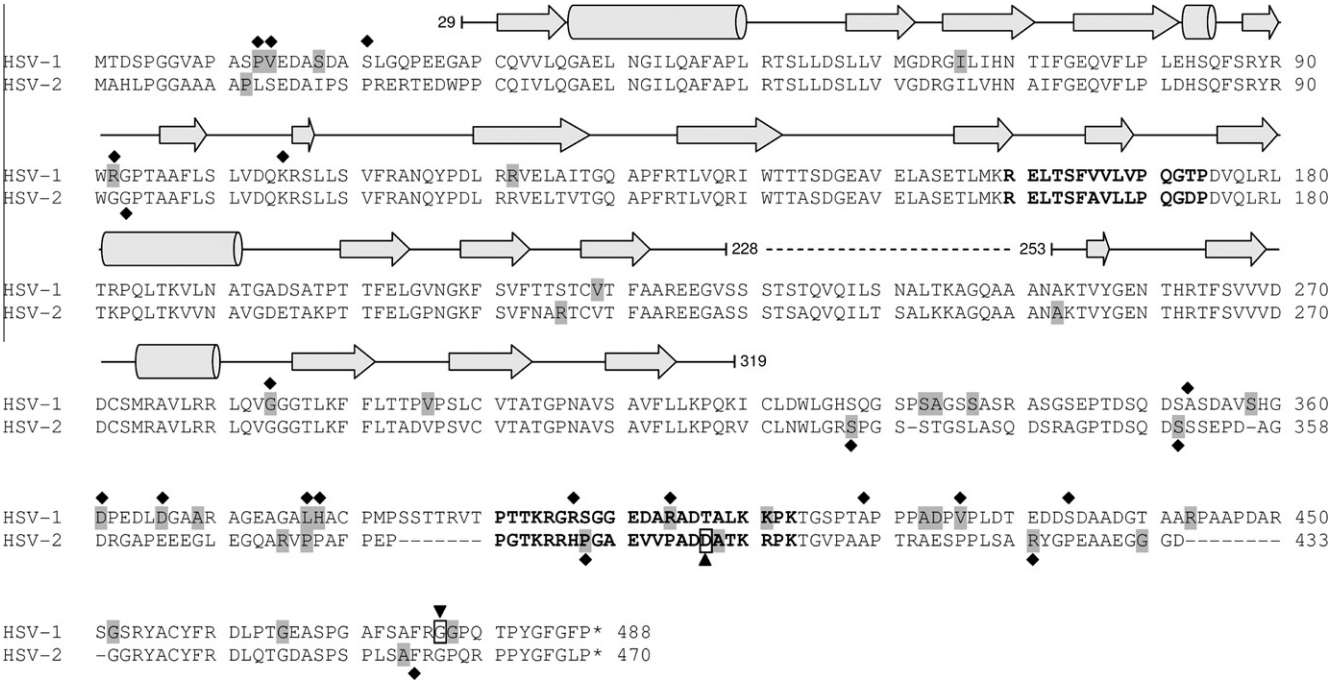

Fig. 1. Natural polymorphism map of UL42 processivity factor among HSV-1 and HSV-2 strains. The alignment of reference strains 17 (HSV-1, above) and HG52 (HSV-2, below) UL42 amino acid sequences (GenBank accession numbers are X14112 and Z86099, respectively) (McGeoch et al., 1985, 1987) was performed using ClustalW software. Assumed conserved regions and functional domains are indicated in bold within the alignment: the connector loop (codons 160–175) (Zuccola et al., 2000) and the nuclear localization signal (NLS, codons 391–413 for HSV-1 and codons 382–404 for HSV-2) (Alvisi et al., 2008). Succession of β-strands (arrows) and α-helix (cylinders) represents three-dimensional structure aligned with UL42 amino acid sequences. Amino acid changes related to natural polymorphism are highlighted in gray, whereas black diamonds represent changes reported in drug-resistant HSV clinical isolates. Frameshift deletions are outlined in black and marked with triangles.

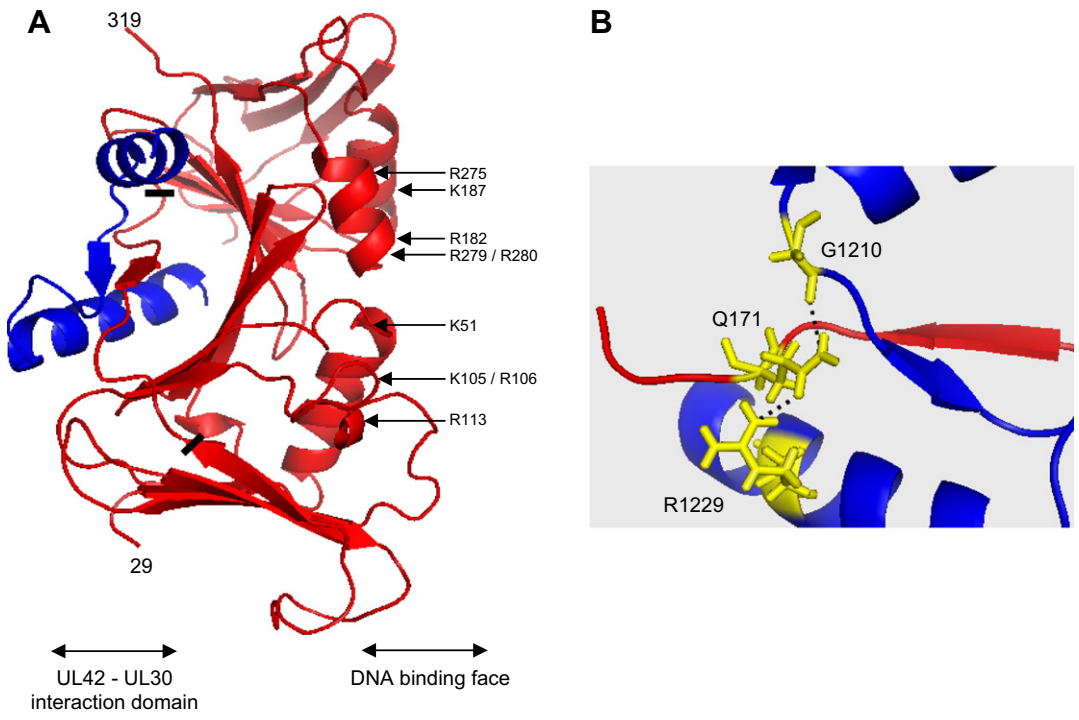

Fig. 2. Protein–protein interaction between UL42 processivity factor and UL30 DNA polymerase of HSV. (A) stereoscopic ribbon diagram of the UL42 (red)/UL30 (blue) complex (Protein Data Bank [PDB] accession number 1dml (Zuccola et al., 2000)). The last ordered residue at the N- and C- termini of each polypeptide is indicated. The black lines indicate both extremities of the UL42 connector loop (residues 160–175) (Zuccola et al., 2000). The UL42 processivity factor harbors a basic surface that contacts DNA on the side opposite to the UL30 binding site (back face). Conserved basic residues involved in DNA binding are indicated sideline (Komazin-Meredith et al., 2008). (B) Representation of the intermolecular hydrogen bonds between Q171 residue from UL42 processivity factor and G1210 and R1229 residues from UL30 DNA polymerase (Loregian and Palu, 2005; Zuccola et al., 2000). For clarity, the side chains of concerned residues are shown in yellow. In the inset, the UL42 connector loop is represented in red and the UL30 peptide backbone is represented in blue.

Acknowledgements

We thank Nathalie Ham and Delphine Voujon for helpful advice and excellent technical assistance. This work was supported in part by the Association pour la Recherche sur les Infections Virales (ARIV).

References

Alvisi, G., Avanzi, S., Musiani, D., Camozzi, D., Leoni, V., Ly-Huynh, J.D., Ripalti, A., 2008. Nuclear import of HSV-1 DNA polymerase processivity factor UL42 is mediated by a C-terminally located bipartite nuclear localization signal. Biochemistry 47, 13764–13777.

Bonnafous, P., Verbelen, M., Petrella, S., Deback, C., Gautheret-Dejean, A., Boutolleau, D., Naesens, L., Agut, H., 2010. Conservation of HHV-6 DNA polymerase processivity factor sequence and predicted structure suggests it as a target for antiviral development. Antiviral Res. 86, 316–319.

Boutolleau, D., Deback, C., Bressollette-Bodin, C., Conan, F., Ait-Arkoub, Z., Imbert-Marcille, B.M., Agut, H., 2009. Genetic analysis and putative role in resistance to antivirals of the human cytomegalovirus DNA polymerase UL44 processivity factor. Antivir. Ther. 14, 847–852.

Burrel, S., Deback, C., Agut, H., Boutolleau, D., 2010. Genotypic characterization of UL23 thymidine kinase and UL30 DNA polymerase of clinical isolates of herpes simplex virus: natural polymorphism and mutations associated with resistance to antivirals. Antimicrob. Agents Chemother. 54, 4833–4842.

Chiba, A., Suzutani, T., Saijo, M., Koyano, S., Azuma, M., 1998. Analysis of nucleotide sequence variations in herpes simplex virus types 1 and 2, and varicella-zoster virus. Acta Virol. 42, 401–407.

Dignani, M.C., Mykietiuk, A., Michelet, M., Intile, D., Mammana, L., Desmery, P., Milone, G., Pavlovsky, S., 2002. Valacyclovir prophylaxis for the prevention of Herpes simplex virus reactivation in recipients of progenitor cells transplantation. Bone Marrow Transplant. 29, 263–267.

Englund, J.A., Zimmerman, M.E., Swierkosz, E.M., Goodman, J.L., Scholl, D.R., Balfour Jr., H.H., 1990. Herpes simplex virus resistant to acyclovir :A study in a tertiary care center. Ann. Intern. Med. 112, 416–422.

Gao, M., DiTusa, S.F., Cordingley, M.G., 1993. The C-terminal third of UL42, a HSV-1 DNA replication protein, is dispensable for viral growth. Virology 194, 647–653.

Gilbert, C., Bestman-Smith, J., Boivin, G., 2002. Resistance of herpesviruses to antiviral drugs: clinical impacts and molecular mechanisms. Drug Resist. Update. 5, 88–114.

Hill, E.L., Hunter, G.A., Ellis, M.N., 1991. In vitro and in vivo characterization of herpes simplex virus clinical isolates recovered from patients infected with human immunodeficiency virus. Antimicrob. Agents Chemother. 35, 2322–2328.

Komazin-Meredith, G., Santos, W.L., Filman, D.J., Hogle, J.M., Verdine, G.L., Coen, D.M., 2008. The positively charged surface of herpes simplex virus UL42 mediates DNA binding. J. Biol. Chem. 283, 6154–6161.

Loregian, A., Palu, G., 2005. Disruption of the interactions between the subunits of herpesvirus DNA polymerases as a novel antiviral strategy. Clin. Microbiol. Infect. 11, 437–446.

Loregian, A., Piaia, E., Cancellotti, E., Papini, E., Marsden, H.S., Palu, G., 2000. The catalytic subunit of herpes simplex virus type 1 DNA polymerase contains a nuclear localization signal in the UL42-binding region. Virology 273, 139–148.

McGeoch, D.J., Dolan, A., Donald, S., Rixon, F.J., 1985. Sequence determination and genetic content of the short unique region in the genome of herpes simplex virus type 1. J. Mol. Biol. 181, 1–13.

McGeoch, D.J., Moss, H.W., McNab, D., Frame, M.C., 1987. DNA sequence and genetic content of the HindIII l region in the short unique component of the herpes simplex virus type 2 genome: identification of the gene encoding glycoprotein G, and evolutionary comparisons. J. Gen. Virol. 68, 19–38.

Randell, J.C., Komazin, G., Jiang, C., Hwang, C.B., Coen, D.M., 2005. Effects of substitutions of arginine residues on the basic surface of herpes simplex virus UL42 support a role for DNA binding in processive DNA synthesis. J. Virol. 79, 12025–12034.

Zuccola, H.J., Filman, D.J., Coen, D.M., Hogle, J.M., 2000. The crystal structure of an unusual processivity factor, herpes simplex virus UL42, bound to the C terminus of its cognate polymerase. Mol. Cell 5, 267–278.