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*In Vitro* Selection of an Herpes Simplex Virus Type 1 (HSV-1) Mutant Resistant to the N7-Isomeric Acyclic Nucleoside Analogue 2242

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The HSV-1 KOS strain was passaged in Vero cells in the presence of gradually increasing concentrations of 2-amino-7-(1,3-dihydroxy-2-propoxymethyl)purine (2242). The 2242-resistant HSV-1 thus obtained (designated $2242^r$) showed cross-resistance to foscarnet (PFA), phosphonoacetic acid (PAA) and the phosphonylmethoxyethyl derivatives of adenine (PMEA) and 2,6-diaminopurine (PMEDAP). In addition, the $2242^r$ strain showed a decreased sensitivity to acyclovir (ACV) and arabinosylcytosine (ara-C), but it remained sensitive to fluoroiodoarabinosylcytosine (FIAC), vidarabine (ara-A), ganciclovir (GCV), bromovinyldeoxyuridine (BVDU) and the 3-hydroxy-2-phosphonylmethoxypropyl derivative of adenine (HPMPA). The $2242^r$ strain behaved similarly to drug-resistant strains obtained upon selection with PFA, PMEA and PMEDAP, since the $PFA^r$, $PMEA^r$ and $PMEDAP^r$ strains were still sensitive to BVDU, FIAC, GCV, ara-A and HPMPA. In addition, the $PFA^r$, $PMEA^r$ and $PMEDAP^r$ strains showed a 5- to 15-fold increase in the $IC_{50}$ for compound 2242. In contrast, $ACV^r$ and $BVDU^r$ HSV-1 strains remained fully sensitive to PFA, PMEA, PMEDAP and compound 2242, while they showed cross-resistance to drugs (i.e. GCV and FIAC) depending on the virus-induced thymidine kinase for their antiviral activity. These results point to similarities in the mechanism of action of compound 2242, PFA, PMEA and PMEDAP.

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*In vitro* Activity of the Herpes Simplex Virus Type 1 and Cytomegalovirus Proteases with Peptide Substrates.

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A viral protease encoded by the UL26 gene of Herpes simplex virus type-1 (HSV-1) protease is responsible for proteolytic processing of itself and the nucleocapsid associated protein, ICP35 (infected cell protein 35) [Liu and Roizman (1991) *J. Virol.* **65**, 5149-5156]. We have identified the HSV-1 cleavage sites between Ala/Ser residues at positions 247/248 and 610/611 in the 635 amino acid protease and have determined that the catalytic domain maps to the 247 amino acids of UL26. This report describes the *in vitro* cleavage of peptides that correspond to each of these cleavage sites by recombinant HSV protease. Analysis of the products by FAB mass spectrometry confirmed that cleavage occurred at the expected position between the Ala and Ser residues of the substrate. A peptide which spans Ala99/Ser100 of the protease, was not a substrate for the protease *in vitro*, confirming that sequence elements outside the conserved dipeptide sequence are required for substrate recognition. Earlier studies identified P5P8' as the minimal substrate peptide for the HSV Ala610/Ser611 cleavage site and revealed a requirement for residues flanking the conserved core P4-LVNA/S-P1' in substrate recognition and hydrolysis. Kinetic analysis with peptide P5P8' yielded a $K_m$ of 190 μM and $k_{cat}$ of 0.2 $min^{-1}$. Additional experiments describing the minimal sequence requirements for the Ala 247/Ser248 cleavage site in HSV will be presented. Open reading frames of HSV-1 UL26 and HCMV UL80 have been reported to have homology. Purified HCMV protease expressed as a glutathione-S-transferase-CMV fusion protein was also found to cleave substrates specific to the HCMV N- and C-terminal cleavage sites. Parallel experiments to identify and compare the minimal peptide requirements and kinetic parameters for HCMV will be discussed.