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Purification and Photoaffinity Labeling of Herpes Simplex Virus Type-1 Thymidine Kinase T. M. Rechtin#, M. E. Black^, F. Mao#, and R.R. Drake#. Department of Biochemistry and Molecular Biology#, University of Arkansas for Medical Sciences, Little Rock, AR 72205 and Darwin Molecular Corporation^, Bothell, WA 98021

The treatment of human herpesviruses with nucleoside drugs is dependent upon phosphorylation by virally-encoded thymidine kinases. Initial phosphorylation by the viral thymidine kinases is neccessary for further metabolism of nucleoside drugs to their active nucleoside triphosphate form. In order to better understand the structural and enzymatic mechanisms by which herpesvirus thymidine kinases recognize nucleoside(-tide) substrates, photoaffinity labeling with [α-^{32}P]5N_3dUMP, [γ-^{32}P]8N_3ATP and [γ-^{32}P]2N_3ATP was used to characterize the thymidine, thymidylate and ATP active-sites of the HSV-1 thymidine kinase. For this study, HSV-1 thymidine kinase (TK) and a mutant enzyme, C336Y (known to confer acyclovir resistance), were bacterially expressed and purified by a rapid, two-step purification procedure independent of thymidine affinity chromatography. This is advantageous for the purification of mutant TK enzymes with altered thymidine affinities. The specificity of photoaffinity labeling of these HSV-1 thymidine kinases was demonstrated by saturation of photoinsertion and by the ability of site-specific substrates such as thymidine, thymidylate, acyclovir, BVDU, and ATP to inhibit photoinsertion. Differences in inhibition patterns of photoaffinity labeling correlated with kinetic differences between the wild-type and C336Y HSV-1 thymidine kinases. Cumulative results suggest that the acyclovir-resistant C336Y TK primarily affects the ATP binding site leading to alteration in the binding affinity of nucleoside drugs in the thymidine site. Photolabeling studies of the ATP binding site in both TKs indicated that the CTP binding site (CTP is an alternative phosphoryl donor) may be distinct from ATP. In this study, azido-nucleotide photoaffinity analogs are shown to be effective tools for studying the active site environment of HSV-1 TK and related mutant TKs. This specific active-site photoincorporation will be exploited for the isolation and identification of crosslinked peptides and amino acids in these HSV-1 TKs.

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Improved Purification of the HSV Protease Catalytic Domain using Immunoaffinity: G. McKercher, D. Thibeault, P.R. Bonneau, R.L. Krogsrud, C. Lawetz, L. Lagacé, M.-J. Massariol, M.G. Cordingley. Bio-Méga/Boehringer Ingelheim Recherche Inc., 2100 rue Cunard, Laval, Québec, Canada H7S 2G5.

Herpes simplex virus protease is essential for maturation of viral capsids. Mutant viruses lacking a functional enzyme cannot package DNA and fail to produce infectious virions. As part of our investigation of HSV protease, we have expressed its catalytic domain in baculovirus-infected cells and produced a panel of specific monoclonal antibodies. The antibodies were evaluated in ELISA, Western blot and enzyme neutralization assays with purified protease *in vitro*. Surface plasmon resonance studies were used to identify two antibodies (3A2 and 6H4) recognizing different epitopes and possessing $\approx$ 10nM K_d. These candidate antibodies were evaluated as ligands for immunoaffinity chromatography. Elution of active protein from antibody 6H4 was achieved with glycine buffer pH 3.5 or 2M $MgCl_2$ whereas efficient elution of protease from 3A2 required much harsher conditions. pH elution from an affinity column of 6H4 was the method of choice for purification since subsequent neutralization was effected more rapidly than dialysis, thus better preserving enzyme activity. Three types of resins were evaluated for antibody immobilization: Affi-Gel Hz, Affi-Gel 10 and Emphaze. Passage of crude lysates through the affinity column produced material of purity and specific activity comparable to preparations of HSV protease purified by a Q-sepharose, Biogel-P60, and MONO-S column sequence. This affinity purification step has allowed us not only to increase the final purity of the enzyme but also to eliminate contaminants that affected the enzyme stability when prepared by previous protocols.