In-Vitro Synthesis of Functional Varicella Zoster and Herpes Simplex Viral Thymidine Kinase

RAVI MAHALINGAM,1 GARY CABIRAC,1 MARY WELLISH,1 DONALD GILDEN,1 AND ABBAS VAFAI1,2
Departments of Neurology1 and Microbiology and Immunology2, University of Colorado Health Sciences Center, Denver, CO 80262

Received August 13, 1989
Revised and accepted November 26, 1989

Requests for reprints should be addressed to Ravi Mahalingam, Department of Neurology, University of Colorado Health Sciences Center, Denver, CO 80262, USA.

Key words: HSV-1 and VZV thymidine kinase, in vitro transcription-translation

Abstract

The varicella-zoster virus (VZV) and herpes simplex virus type 1 (HSV-1) thymidine kinase (TK) genes were cloned into the transcription vector pGEM4. In-vitro translation (ivt) of RNA transcribed from these genes showed prominent expression of functional TK proteins with the expected molecular weights of 36 kD for VZV and 43, 39, and 38 kD for HSV-1. The TK proteins were recognized by rabbit anti-VZV and anti-HSV-1 antibodies, respectively. Analysis of the ivt products by thin-layer chromatography revealed the conversion of thymidine to its phosphorylated forms (TMP, TDP, and TTP) by both the VZV and HSV-1 TK genes. The estimated specific activities of the in-vitro translated VZV and HSV-1 TKs were comparable. VZV TK templates were linearized at internal restriction sites and RNAs transcribed from these templates directed the synthesis of polypeptides with sizes consistent with the colinearity of the VZV TK gene. Deletion of 107 amino acids at the carboxy terminus of the VZV TK gene abolished the in-vitro TK activity. In addition, immunoprecipitation of truncated proteins synthesized in vitro suggested the possible involvement of the region between amino acid residues 101 and 168 from the amino terminus of the VZV TK molecule in the formation of structures necessary for antigenicity.

Introduction

Varicella-zoster virus (VZV) and herpes simplex virus type 1 (HSV) induce pyrimidine deoxyribonucleotide kinase (thymidine kinase, TK) in productively in-
fected cells (1–5). The induced TK enzyme has been shown to be virus encoded and different from the cellular TK (6,7). HSV-1 TK expression has also been shown to be important for the establishment of latency (8,9), and it has been hypothesized that a host-dependent latency function may be encoded in the domain of the HSV-1 TK gene (8). However, there is no information on the role of VZK TK in the establishment of latency. Both viral TKs convert thymidine to thymidine 5' monophosphate (TMP) and TMP to thymidine 5' diphosphate (TDP) (10). TDP is further phosphorylated by a host-specific kinase to thymidine 5' triphosphate (TTP), which in turn is used by the viral DNA polymerase for the synthesis of the viral DNA (11). VZV and HSV-1 TK have also been shown to catalyze preferentially the phosphorylation of deoxycytidine over the host TK (3,5,6,11).

The VZV TK gene (gene 36), located between nucleotides 64807 and 65829 of the VZV genome (12), encodes a primary translation product of 35,000 (35 kD), which may also exist as a dimer (13,14). The HSV-1 TK gene (UL23 gene), which is located between nucleotides 46674 and 47802 of the HSV-1 genome (15), contains three open reading frames (ORFs) encoding polypeptides with apparent molecular weights of 43, 39, and 38 kD (16–18). It has been shown that HSV-1 TK and VZV TK share a limited amino acid similarity (28%) (12,19).

The activity of the viral TK is associated with at least two regions (ATP and thymidine binding sites) of the TK polypeptides (20). Comparison of the predicted amino acid sequences has shown that the ATP and thymidine-binding domains of HSV-1 and VZV display 55% and 64% sequence similarities, respectively (19), suggesting conserved functional domains. Although point mutations at the ATP and thymidine binding sites of VZV TK result in resistance to antiviral agents (19,21), the nature of the interaction between the antiviral agents and the active domain of the viral TK molecule has not been defined. Analysis of HSV-1 TK in vitro indicates that translation of cytoplasmic mRNA specific for HSV-1 TK results in enzymatically active protein (22). Using an in-vitro transcription-translation system to analyze VZV genes (23), we describe here the expression of functional TK from VZV and HSV-1, and the analysis of the functional and antibody-binding domains of VZV TK. The results indicated that a functional viral TK synthesized in vitro is capable of phosphorylating thymidine and that the specific activities of VZV and HSV-1 TK were comparable. Furthermore, our results identified a) an antigenic determinant on the VZV TK polypeptide between amino acid residues 101 and 168 and b) a functional domain at the carboxy terminal region (between amino acid residues 238 and 342) of VZV TK.

Materials and Methods

Cloning of the VZV SalI-A fragment

The VZV SalI-A DNA fragment (33.5 kbp) containing the TK gene was cloned into the cosmid vector pHC79 at the SalI site as described earlier (23).