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Purification of full-length VP22 from cells infected with HSV-1: A two-pronged approach for the solubilization and purification of viral proteins for use in biochemical studies

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VP22, encoded by the U_L49 gene, is one of the most abundant proteins of the herpes simplex virus type 1 (HSV-1) tegument and has been shown to be important for virus replication and spread. However, the exact role(s) played by VP22 in the HSV-1 replication cycle have yet to be delineated. The lack of a procedure to purify full-length VP22 has limited molecular studies on VP22 function. A procedure was developed for the purification of soluble, full-length VP22 from cells infected with HSV-1. A recombinant virus encoding His-tagged VP22 was generated and found to express VP22 at levels comparable to the wild type virus upon infection of Vero cells. By experimenting with a wide variety of cell lysis buffer conditions, several buffers that promote the solubility of full-length VP22 were identified. Buffers that gave the highest levels of solubility were then used in immobilized metal ion affinity chromatography experiments to identify conditions that provided the greatest level of VP22 binding and recovery from cobalt and nickel affinity resins. Using this strategy soluble, full-length VP22 was purified from cells infected with HSV-1.

1. Introduction

Herpes simplex virus type I (HSV-1) is a double-stranded DNA virus that is a member of the *Alphaherpesvirinae* subfamily. HSV-1 virions, like those of all herpesviruses, are composed of an icosahedral capsid encasing the viral genome, a proteinaceous layer termed the tegument that surrounds the nucleocapsid, and a host-derived lipid membrane envelope that contains viral glycoproteins. The tegument contains an estimated 20 viral proteins that are deposited into the host cell along with the nucleocapsid upon infection. Tegument proteins have been shown to play a variety of roles in infection, including the regulation of viral and host gene expression and the promotion of virus assembly and egress (Campbell et al., 1984; Fuchs et al., 2002; Pellett et al., 1985; Read et al., 1993). Though many of the HSV-1 tegument proteins have been well characterized, the functions of others are not yet clear.

Viral Protein 22 (VP22), encoded by the U_L49 gene (Elliott and Meredith, 1992), is a tegument protein that, despite numerous illuminating studies, does not yet have a clearly defined role in the HSV-1 replication cycle. VP22 is phosphorylated, 301 amino acids in length, has an observed molecular weight of 38 kDa, and is largely conserved among the alphaherpesviruses (Elliott and Meredith, 1992; Knopf and Kaerner, 1980). VP22 is one of the most abundant tegument proteins with ~2000 copies present in each virion (Heine et al., 1974; Leslie et al., 1996). A number of interesting properties have been attributed to this protein including association with microtubules (Elliott and O'Hare, 1998; Kotsakis et al., 2001) and association with chromatin (Aints et al., 2001; Elliott and O'Hare, 1997, 1998, 2000; Ingvarsdottir and Blaho, 2010; Knopf and Kaerner, 1980; Kotsakis et al., 2001; Lopez et al., 2008; Martin et al., 2002; van Leeuwen et al., 2003). Additional studies have shown that VP22 promotes viral protein synthesis (Duffy et al., 2009) and viral spread (Duffy et al., 2006; Pomeranz and Blaho, 2000). To define VP22's role(s) in HSV-1 replication, additional molecular and biochemical studies using purified full-length VP22 are required.

Previous attempts to express full-length VP22 in various bacterial systems resulted in very low yields of soluble protein (Normand et al., 2001). As a result, in vitro examinations of VP22 function have been limited to the study of the carboxyl-terminal half of the protein, which is soluble following expression in *Escherichia coli* (Normand et al., 2001; van Leeuwen et al., 2003). Two possible reasons for the insolubility of full-length VP22 in previously-examined expression/purification systems are (1) the overexpression of the protein in a non-native environment and (2) lysis and purification of the protein under sub-optimal buffer conditions. VP22 is known to interact with several different viral proteins during HSV-1 infections. Expression in the context of these interactions may be required for VP22 solubility. To address this concern, a recombinant

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herpesvirus that expresses VP22 with a 6× histidine tag (His-tag) fusion was generated for purification of VP22 via immobilized metal ion affinity chromatography (IMAC) following expression of the protein in its native environment. To identify buffer conditions that promoted VP22 solubility, the sparse matrix approach used by Lindwall et al. (2000) was adapted to screen a wide variety of lysis buffer conditions. Following expression of VP22 via infection of Vero cells with the HSV-1 VP22-His virus, cells were lysed in different buffers that varied in pH, chaotropicity, and solubility additive(s). Following lysis, immunoblots were used to determine the proportion of VP22 that was soluble in each different lysis buffer. Finally, buffer conditions that maximized solubility were compared to identify conditions that also maximized binding and recovery of His-tagged VP22 on cobalt and nickel IMAC resins. Using this method, soluble full-length VP22 was purified to near-homogeneity.

2. Materials and methods

2.1. Viruses and cells

Viral stocks of wild type HSV-1(F) and the HSV-1 VP22-His viruses were propagated exclusively on Vero cells. Vero cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 4.0 mM L-glutamine, 4.5 g/l glucose, 125 units/ml penicillin, 0.125 mg/ml streptomycin, and 10% newborn calf serum. Infections were performed in medium 199 supplemented with 1% newborn calf serum.

2.2. Generation of wild type and VP22-His viruses

Wild type HSV-1(F) (WT) and a virus that encodes VP22 with a C-terminal 6× His-tag fusion (VP22-His) were constructed using the HSV-1(F) bacterial artificial chromosome (BAC) pYEbac102 (Tanaka et al., 2003) (Fig. 1). The U_L49His BAC, in which a DNA sequence encoding a 6× His tag was fused in-frame to the 3′ end of U_L49, was generated according to the method of Tischer et al. (2006) as follows. A linear recombination fragment containing a *kan*^{R} gene and I-*Sce*I endonuclease site flanked by short sequences homologous to regions up- and downstream of the U_L49 stop codon and encoding a His-tag insertion was generated by PCR and gel purified. pLay2, a plasmid containing a *kan*^{R}-I-*Sce*I cassette was used as a template for the PCR reaction. The forward primer used for the PCR reaction was 5′-CCCAGCCCGCTCCGCTTCTCGCCCCAGACGGCCCGTCGAGCATCACCATCACCATCACTGA*AAACTTCCGTACCCAGA***TAGGGATAACAGGGTAATC GATTT**-3′ (Integrated DNA Technologies, Coralville, IA); nucleotides in Roman type are homologous to target sequences upstream of the U_L49 stop codon (HSV-1 (F) bps 105,528-105,489) (McGeoch et al., 1988), underlined nucleotides comprise the His-tag insertion and the U_L49 stop codon, italicized nucleotides are homologous to target sequences downstream of the U_L49 stop codon (HSV-1(F) bps 105,485-105,468), and nucleotides in bold-face are homologous to the *kan*^{R}-I-*Sce*I template cassette of pLay2. The reverse primer was 5′-CCCCTGTTGGTGCTTTATTGTCTGGGTACGGAAGTTTTCAGTGATGGTGATGGTGATG*CTCGACGGGCCCGTCTGGGGC***GCCAGTGTTACAA CCAATTAACC**-3′ (Integrated DNA Technologies, Coralville, IA); nucleotides in Roman are homologous to target sequences downstream of the U_L49 stop codon (HSV-1 (F) bps 105,448-105,485), underlined nucleotides comprise the His-tag insertion and the U_L49 stop codon, italicized nucleotides are homologous to target sequences upstream of the U_L49 stop codon (HSV-1(F) bps 105,489-105,508), and nucleotides in bold-face are homologous to the *kan*^{R}-I-*Sce*I template cassette of pLay2. Recombination-competent *E. coli* GS1783 cells carrying pYEbac102 were transformed via electroporation with the above recombination fragment. *E. coli* GS1783, a kind gift from Gregory Smith, encodes the bacteriophage λ *exo*, *bet*, and *gam* recombination and nuclease-inhibition genes under the control of the λ *cI857* temperature-sensitive repressor and an arabinose-inducible gene encoding the SceI restriction endonuclease (Tischer et al., 2010). Following growth at 30 °C on LB agar plates containing 30 μg/ml chloramphenicol (Cm) and 50 μg/ml kanamycin (Kan), Cm^R-Kan^R recombinants were genotypically verified by restriction fragment length polymorphism (RFLP) of alkaline lysis-extracted BAC DNA. Next, the *kan*^{R} gene was excised through Red-mediated recombination following arabinose-mediated induction of the SceI restriction endonuclease and 42 °C induction of the λ *exo*, *bet*, and *gam* genes. Kanamycin-sensitive clones were identified via replica plating on LB agar plates containing either Cm (30 μg/ml) or Kan (50 μg/ml) and the BACs were again genotypically verified by RFLP of alkaline-lyis extracted BAC DNA. The resulting BAC was named pYEbac102-U_L49His.

WT and VP22-His viruses were generated from pYEbac102 and pYEbac102-U_L49His, respectively, as follows.

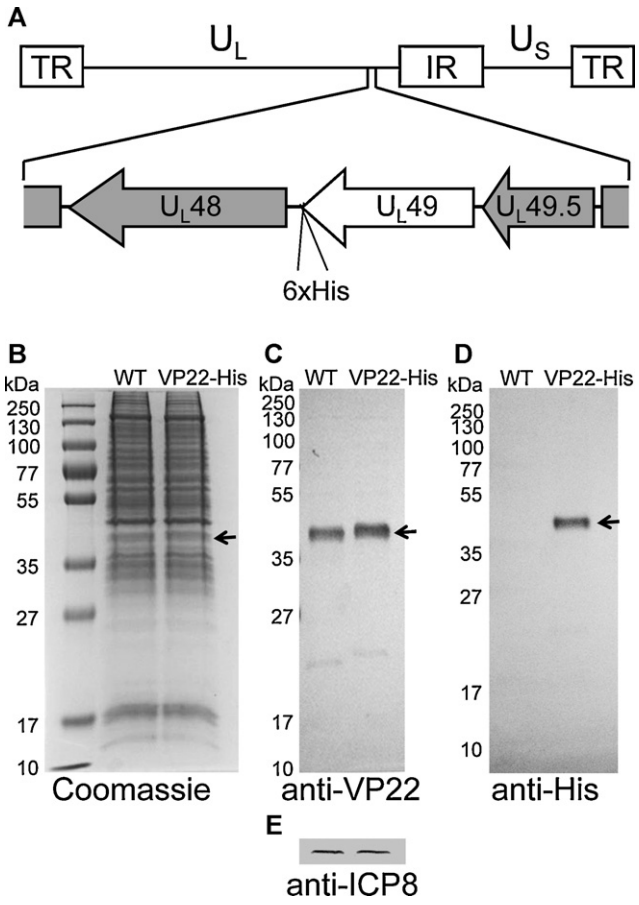

Fig. 1. Generation of HSV-1 VP22-His recombinant virus and expression of His-tagged VP22. (A) Schematic of the VP22-His virus generated using the HSV-1 BAC system. The U_L49 gene is located in the Unique Long (U_L) region of the HSV-1 genome. Nucleic acids encoding six histidine residues were inserted in-frame between the last coding triplet and the stop codon of U_L49. (B) Protein expression during infection with the WT and VP22-His recombinant viruses. Coomassie-stained SDS-PAGE gel of lysates from Vero cells infected with WT HSV-1 or the VP22-His recombinant virus. (C) VP22 expression during infection with the WT and VP22-His recombinant viruses. Immunoblot analysis of lysates from Vero cells infected with WT HSV-1 and VP22-His using an anti-VP22 antibody. (D) Presence of the 6× His tag on VP22 produced during VP22-His recombinant virus infection. Immunoblot analysis of lysates from Vero cells infected with WT HSV-1 and VP22-His using an anti-pentahistidine antibody. (E) ICP8 expression during infection with the WT and VP22-His recombinant viruses. Immunoblot analysis of lysates from Vero cells infected with WT HSV-1 and VP22-His using and anti-ICP8 antibody.