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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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Summary

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.

Keywords

Protein solubility; viral protein purification; HSV-1; VP22

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

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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; Elliott and O'Hare, 1998; Elliott and O'Hare, 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, 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 *E. 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 herpesvirus that expresses VP22 with a 6x 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. (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

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 6x 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 6x His tag was fused in-frame to the 3' end of U_L49, was generated according to the method of Tischer et al. (Tischer et al., 2006) as follows. A linear recombination fragment containing a *kan^R* gene and I-*SceI* 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-*SceI* cassette was used as a template for the PCR reaction. The forward primer used for the PCR reaction was 5'-
CCCAGCCCCGCTCCGCTTCTCGCCCCAGACGGCCCGTCGAGCATCACCATCACCA
TCACTGAAAACTTCCGTACCCAGATAGGGATAACAGGGTAATCGATTT-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 105528-105489) (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 105485-105468), and nucleotides in bold-face are homologous to the *kan^R*-I-*SceI* template cassette of pLay2. The reverse primer was 5'-
CCCCTGTTGGTGCTTTATTGTCTGGGTACGGAAGTTTTTCAGTGATGGTGATGGTG
ATGCTCGACGGGCGCTCTGGGGCGCCAGTGTTACAACCAATTAACC-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 105448-105485), 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 105489-105508), and nucleotides in bold-face are homologous to the *kan^R*-I-*SceI* 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 μ g/ml Cm and 50 μ g/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 μ g/ml) or Kan (50 μ g/ml) and the BACs were again genotypically verified by RFLP of alkaline-lysis 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. Twenty-five-cm² flasks of Vero cells at ~80% confluency were co-transfected with ~300 ng alkaline lysis-extracted BAC DNA and ~2.5 μ g of a Cre recombinase expression vector (pCAGGS-nlsCre, a gift from Michael Kotlikoff, Cornell University) using Superfect transfection reagent (Qiagen, Valencia, CA). Cre recombinase expressed in BAC transfected cells mediated excision of the BAC vector sequences via recombination between the flanking loxP sites. Viral plaques were purified and propagated on Vero cells.

2.3 Analysis of VP22-His protein expression

Approximately 1.2×10^6 Vero cells were infected for 13 h with either the WT or VP22-His viruses. Infected cells were scraped into the overlying media and collected by centrifugation. Infected cell pellets were resuspended in 100 μ l SDS-PAGE sample buffer (100 mM Tris [pH 6.8], 200 mM DTT, 4% SDS, 0.2% bromophenol blue, 20% glycerol), boiled for 5 min and briefly sonicated to shear genomic DNA. Proteins were separated by 12% SDS-PAGE and either stained with Coomassie Brilliant Blue or transferred to a nitrocellulose membrane for immunoblot analysis. VP22 was visualized via immunoblot using a polyclonal anti-VP22 antibody produced by Aves Labs. To compare VP22 expression in Vero cells infected with the wild type and VP22-His viruses, the immunoblots were stripped and re-probed with an antibody specific to the HSV-1 ICP8 protein (Shelton et al., 1994). For each virus, the VP22 and ICP8 signals were quantified and the ratio of VP22 signal to ICP8 signal was calculated. Immunoblots were also performed using a monoclonal anti-pentahistidine antibody (Sigma Aldrich, St. Louis, MO) to ensure the VP22 C-terminal His-tag was not cleaved during infection.

2.4 Identification of buffer conditions that maximize VP22 solubility

Four T175 flasks of Vero cells were grown to 90-95% confluence, infected with the VP22-His virus at an MOI of 5, and collected at 6 hours post-infection (hpi) via centrifugation. Infected cell pellets were combined, resuspended in 50 ml of phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 , 2 mM KH_2PO_4) and divided into 1 ml aliquots. Cell aliquots were centrifuged to pellet the infected cells. Infected cell pellets were frozen at -80°C until later use.

To identify buffer conditions that promote the solubility of full-length VP22, each of the various buffers in Figure 2 was tested using the following protocol. A frozen pellet of infected cells was resuspended in 1.5 ml of a chosen buffer plus 20 $\mu\text{L}/\text{ml}$ protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO) and incubated on ice for 15 min. A 100- μl sample of this cell lysate was taken, mixed with 50 μl SDS-PAGE sample buffer, and boiled for 5 min. This sample is referred to as the “total lysate” (TL) in figure 2. The remaining 1.4 ml of cell lysate was centrifuged for 30 min at 4°C and $10,000 \times g$. The supernatant containing soluble proteins was removed to another tube on ice. A 100 μl sample of this soluble fraction was mixed with 50 μl SDS-PAGE sample buffer and boiled for 5 min. This sample is referred to as the “soluble fraction”. The pellet containing insoluble proteins was resuspended in a volume of the same lysis buffer equal to that of the soluble fraction. A 100- μl sample of this insoluble fraction was mixed with 50 μl SDS-PAGE sample buffer and boiled for 5 min. This sample is referred to as the “insoluble fraction”. Proteins comprising the total lysate, soluble fraction, and insoluble fraction were separated by 12% SDS-PAGE and analyzed via immunoblot using an anti-VP22 antibody. Qualitative comparisons of VP22 present in the soluble versus insoluble fractions were used to identify buffer conditions that promote the solubility of full-length VP22.

2.5 Purification of VP22-His from cells infected with HSV-1 VP22-His

Vero cells grown to 90-95% confluence were infected with the HSV-1 VP22-His virus at an MOI of 5 PFU/ml. Infected cells were collected at 13 hpi by removing the media and scraping the cells into chilled PBS. Infected cells were pelleted by centrifugation at 1200 rpm for five min at 4°C . The supernatant was removed and the remaining pellet was frozen at -80°C until purification.

The frozen, infected cell pellet was resuspended in lysis buffer consisting of 100 mM HEPES (pH 7.5), 1 M MgSO_4 , 20 mM imidazole, 1% NP-40 and 20 $\mu\text{l}/\text{ml}$ protease inhibitor cocktail (Sigma Aldrich, St. Louis, MO). Resuspended cells were sonicated briefly then held

on ice for 45 min. At this point a sample was taken for future analysis (referred to as “total lysate” in figure 3). The cell lysate was centrifuged for 1 h at 4°C and $18,000 \times g$ to separate soluble and insoluble cellular components. The soluble fraction was decanted and syringe filtered first using a 0.80 μm filter then a 0.45 μm filter. A sample was taken of the filtered, soluble fraction for future analysis (referred to as “soluble” in figure 3). The filtered, soluble fraction was mixed with 1 ml of pre-equilibrated Talon cobalt resin (Clontech Laboratories, Mountain View, CA). Binding of His-tagged VP22 to the resin took place via rocking at 4°C for 2 h. The resin was then washed 3 times by rocking in each of the following wash buffers for 20 min at 4°C followed by centrifugation and removal of the supernatant. Wash buffer 1 = 100 mM HEPES (pH 7.5), 1 M MgSO_4 , 40 mM imidazole, 200 mM NaCl; Wash buffer 2 = 100 mM HEPES (pH 7.5), 1 M MgSO_4 , 40 mM imidazole, 600 mM NaCl; Wash buffer 3 = 100 mM HEPES (pH 7.5), 1 M MgSO_4 , 40 mM imidazole. The unbound proteins removed prior to wash 1 are referred to as the “flow-through” in figure 3. Following the last wash, the resin was transferred to a disposable 5 mL column for elution. The bottom of the column was capped and 1 ml of elution buffer 1 (100 mM HEPES [pH 7.5], 1 M MgSO_4 , 100 mM imidazole) was added to the resin and held on ice for 30 min. The column was then uncapped and drained into a collection tube (referred to as “eluate 1” in figure 3). The elution process was repeated with elution buffer 2 (100 mM HEPES [pH 7.5], 1 M MgSO_4 , 250 mM imidazole, 1% NP-40) (referred to as “eluate 2” in figure 3). The second elution, containing purified VP22, was dialyzed against 1.5 L dialysis buffer 1 (100 mM HEPES [pH 7.5], 500 mM MgSO_4) for 18 hr., then against 1.5 L dialysis buffer 2 (100 mM HEPES [pH 7.5], 200 mM MgSO_4) for 18 hr., then against storage buffer (20 mM HEPES [pH 7.5], 150 mM MgSO_4 , 1 mM DTT, 10% glycerol) for 18 h. Purified VP22 was stored at -80°C . All samples were analyzed via 12% SDS-PAGE and either stained with Coomassie Brilliant Blue or immunoblotted with an anti-VP22 antibody to verify the presence of VP22.

2.6 SDS-PAGE and Immunoblot

Protein samples and cell fractions were mixed with SDS-PAGE sample buffer and separated by 12% denaturing SDS-PAGE at 135 V for 1.5 h. Gels were stained with Coomassie brilliant blue (Bio-Rad, Hercules, CA) to visualize proteins. Alternatively, proteins were transferred to a nitrocellulose membrane using a wet electroblotting system (Bio-Rad, Hercules, CA). Membranes were blocked overnight at 4°C in 5% (w/v) skim milk in PBS and then washed by rocking in PBS for 10 min at room temperature. Membranes were rocked overnight at 4°C with primary antibodies diluted 1:2000 in 10% (w/v) bovine serum albumin in PBS. Following three 10-min washes in PBS-T (0.1% Tween-20 in PBS), membranes were rocked for 2 h at room temperature with secondary antibodies conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:500 in 10% (w/v) bovine serum albumin in PBS. After three 10-min washes in PBS-T, membranes were developed with either the CN/DAB Substrate Kit (Thermo Scientific, Rockford, IL) for direct visualization or the Pierce ECL Western Blotting Substrate (Thermo Scientific, Rockford, IL) followed by autoradiography and chemiluminescence quantification.

3. Results

3.1 Generation of the VP22-His recombinant virus

To determine whether soluble, full-length VP22 could be purified from cultured cells infected with HSV-1, a recombinant virus that encodes VP22 as a carboxyl-terminal His-tag fusion protein was generated using the HSV-1 BAC system as described in Materials and Methods. The VP22-His recombinant virus encodes six histidines between the last coding nucleotide and the stop codon of U_L49 (Fig. 1A). To compare expression of VP22 in cells infected with the VP22-His virus to expression in cells infected with the wild type virus, the

HSV-1 BAC system was also used to generate a BAC-derived wild type virus (WT) as described in Materials and Methods. To verify the expected genotype of the VP22-His recombinant virus, viral DNA was purified and the genetically manipulated region of the genome was amplified by PCR and sequenced. All nucleotide sequences were found to be correct as designed (data not shown).

To determine whether the carboxyl-terminal His-tag affected the expression of VP22 in infected cells, lysates of Vero cells infected with either the wild type or VP22-His virus for 13 hours were analyzed via SDS-PAGE and immunoblotting (Figs. 1B-1E). Coomassie Brilliant Blue staining following SDS-PAGE showed the protein profiles produced in cells infected with the wild type and VP22-His viruses were not noticeably different (Fig. 1B). Immunoblot analysis was performed using an anti-VP22 polyclonal antibody (Fig. 1C), followed by stripping of the membrane and re-probing with an antibody specific to the HSV-1 ssDNA binding protein ICP8 (Fig. 1E). The VP22 and ICP8 signals were quantified and VP22 to ICP8 signal ratios were calculated for each virus. The VP22 to ICP8 signal ratio was 3.6 for the wild type virus and 3.2 for the VP22-His virus showing relative expression of VP22 is very similar in cells infected with either of the two viruses. These data indicate the carboxyl-terminal His-tag does not affect expression of VP22 during infection.

The efficiency of protein purification can be adversely affected by the *in vivo* cleavage of the His-tag from His-tag fusion proteins. To verify the presence of the His-tag on VP22 produced during VP22-His infection, immunoblot analysis was performed using an anti-pentahistidine antibody (Fig. 1D). A strong band was visible in the lane containing the VP22-His lysate, but not in the wild type lysate. Although it is not known whether the His-tag was retained on every molecule of VP22 synthesized during VP22-His infection, enough was retained to warrant continuation of the purification strategy.

3.2 Optimization of buffer conditions for the purification of soluble VP22

To identify lysis buffer conditions that promote the solubility of full-length VP22, a sparse matrix approach was utilized. Different buffers of varying pH were mixed and matched with different salts of varying chaotropicity and different solubility additives. Thirty-five different buffers were analyzed for their effects on VP22 solubility (Fig. 2). Briefly, Vero cells infected with the VP22-His recombinant virus were collected, washed, aliquoted, pelleted, and frozen. Individual cell pellets were then resuspended in each of the lysis buffers listed in figure 2. A sample was taken of the total cell lysate, and the remaining lysate was centrifuged to separate soluble and insoluble proteins. The soluble fraction was removed to a clean tube and the insoluble pellet was resuspended in a volume of lysis buffer equal to that of the soluble fraction. Samples of the soluble and resuspended insoluble fractions were taken and, together with the total lysate, were separated by SDS-PAGE. Anti-VP22 immunoblot analysis was performed to qualitatively determine the solubility of full-length VP22 under the various lysis buffer conditions. Buffers containing 1 M MgSO₄ were found to promote the highest levels of VP22 solubility. For example, approximately 50% of VP22 was soluble in buffer 12 (100 mM HEPES, 1 M MgSO₄, 1% NP-40, pH 7.5) and approximately 70%-90% of VP22 was soluble in buffers 17-23 (100 mM NaOAc, 1 M MgSO₄, 1% NP-40, pH 5.0-7.5).

To determine which of the MgSO₄-containing buffers promoted the highest efficiency of VP22 purification, additional studies were performed. Vero cells infected with the VP22-His virus were lysed in buffers 12, 22, and 23 and the soluble fractions were mixed with cobalt IMAC resin. Following binding and washing, a sample of the resin was taken to examine binding efficiency. Bound proteins were eluted twice with imidazole and a sample of each eluate was taken to examine elution efficiency. The highest yields of VP22 were obtained following lysis in buffer 12 (data not shown). Next, nickel and cobalt IMAC resins were

compared for VP22 purification efficiency. The soluble fraction from cells infected with VP22-His and lysed in buffer 12 were mixed with either a nickel resin or a cobalt resin. Following binding, washing, and elution, it was found that the cobalt resin promoted a higher level of protein purity (data not shown).

3.3 Purification of VP22 from cells infected with the VP22-His virus

A one-step cobalt immobilized metal affinity chromatography procedure, as described in Materials and Methods, was used to purify VP22 from Vero cells infected with the VP22-His virus. This simple process yielded VP22 of very high purity in the second elution as observed by the single Coomassie Brilliant Blue-stained band in eluate 2 (Fig. 3A). The identity of the purified protein was verified by immunoblot analysis using an anti-VP22 antibody (Fig. 3B). Using the described method, 1.2 mg of soluble, full-length VP22 can be obtained from 2.5×10^9 infected Vero cells.

4. Discussion

In this study, a method for the expression and purification of soluble, full-length VP22 is reported. The approach involved the purification of VP22 from Vero cells infected with a recombinant HSV-1 that encodes VP22 with a carboxy-terminal 6x histidine tag. Although typical recombinant protein yields are generally much higher following over-expression in *E. coli*, use of this method for the overexpression of full-length VP22 has previously resulted in highly insoluble protein (Normand et al., 2001). Our method provides highly pure, soluble, full-length VP22 in quantities adequate for *in vitro* biochemical assays of interest to VP22 researchers. To our knowledge, this is the first report of successful expression and purification of soluble, full-length VP22.

The approach to the purification of soluble, full-length VP22 was two-pronged. First, it was hypothesized that overexpression of VP22 may lead to decreased solubility. It was also hypothesized that co-expression with other HSV-1 proteins may be necessary for the correct folding and solubility of VP22. Therefore, the reported approach aimed to maximize VP22 solubility by expressing the protein to its natural intracellular level and in its natural environmental context as part of the HSV-1 infection cycle. Because VP22 is an abundant HSV-1 protein, and is naturally expressed at high levels in cells infected with HSV-1, no changes were made to the VP22 promoter in the VP22-His recombinant virus. However, the reported method can be applied to less abundant viral proteins by placing a highly active promoter in front of the gene of interest during recombinant virus generation.

Second, while typical protein purification protocols call for the use of lysis buffers that mimic 'ideal', or average, intracellular conditions (i.e. pH near 7.0 and 100-150 mM NaCl), it has long been known that proteins exist within living cells in localized conditions that can vary widely from this average (Minton, 1981). These 'non-ideal' conditions can be mimicked successfully with buffers that contain 'non-physiological' salts and additives (Lindwall et al., 2000; Minton and Wilf, 1981). Therefore, the solubility of full-length VP22 in a range of buffers that varied in pH, chaotropicity, and solubility additive(s) was examined. Using this method, buffer conditions were identified that allowed for the successful purification of soluble, full-length VP22.

By combining natural expression conditions with optimal lysis buffer conditions a method was developed for the successful purification of a previously troubling viral protein. This method will be of use in the HSV-1 field for the further study of VP22 structure and function. In addition, this approach can be applied to other systems for the successful purification of viral proteins that are insoluble under standard expression and purification conditions.

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- A recombinant HSV-1 that encodes VP22 as a His-tag fusion protein was generated
- The C-terminal His-tag did not affect expression of VP22 during HSV-1 infection
- Optimal buffer conditions for VP22 solubility were identified
- Soluble, full-length VP22 was purified from cells infected with HSV-1

Figure 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 6x 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 anti-ICP8 antibody.

Immunoblots			Buffer Composition
TL	Sol	Insol	
			1. 100 mM Tris, 10% glycerol, 1% NP-40, pH 7.5
			2. 100 mM Tris, 50 mM LiCl, 0.5% NP-40, pH 7.5
			3. 100 mM HEPES, 50 mM (NH ₄) ₂ SO ₄ , 10% glycerol, 0.5% NP-40, pH 7.5
			4. 100 mM HEPES, 100 mM KCl, 1% NP-40, pH 7.5
			5. 100 mM Tris, 50 mM NaCl, 10% Isopropanol, 0.5% NP-40, pH 8.2
			6. 100 mM K ₂ HPO ₄ /K ₂ HPO ₄ , 50 mM (NH ₄) ₂ SO ₄ , 1% Triton X-100, pH 6.0
			7. 100 mM triethanolamine, 100 mM KCl, 10 mM DTT, 0.5% NP-40, pH 8.5
			8. 250 mM KH ₂ PO ₄ /K ₂ HPO ₄ , 0.1% CHAPS, pH 6.0
			9. 100 mM triethanolamine, 50 mM LiCl, 5 mM EDTA, 0.5% NP-40, pH 8.5
			10. 100 mM NaOAc, 100 mM glutamine, 10 mM DTT, 0.5% NP-40, pH 5.5
			11. 100 mM NaOAc, 100 mM KCl, 0.1% n-octyl-β-D-glucoside, pH 5.2
			12. 100 mM HEPES, 1 M MgSO ₄ , 1% NP-40, pH 7.5
			13. 100 mM HEPES, 50 mM LiCl, 0.1% CHAPS, pH 7.5
			14. 100 mM Tris, 50 mM NaCl, 5 mM calcium acetate, 0.5% NP-40, pH 7.5
			15. 100 mM triethanolamine, 50 mM (NH ₄) ₂ SO ₄ , 10 mM MgSO ₄ , 0.5% NP-40, pH 8.5
			16. 100 mM Tris, 100 mM KCl, 2 mM EDTA, 1% Triton X-100, pH 8.2
			17. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 5.0
			18. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 5.2
			19. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 5.5
			20. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 6.0
			21. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 6.5
			22. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 7.2
			23. 100 mM NaOAc, 1 M MgSO ₄ , 0.5% NP-40, pH 7.5
			24. 100 mM Tris, 2 M NaCl, 0.1% n-octyl-β-D-glucoside, pH 7.5
			25. 100 mM Tris, 1 M (NH ₄) ₂ SO ₄ , 10mM DTT, 0.5% NP-40, pH 8.2
			26. 100 mM NaOAc, 50 mM LiCl, 5 mM calcium acetate, 0.5% NP-40, pH 5.2
			27. 100 mM Tris, 50 mM NaCl, 100 mM urea, 0.5% NP-40, pH 8.2
			28. 100 mM triethanolamine, 100 mM KCl, 0.05% dextran sulfate, 0.5% NP-40, pH 8.5
			29. 100 mM KH ₂ PO ₄ /K ₂ HPO ₄ , 50 mM (NH ₄) ₂ SO ₄ , 0.05% dextran sulfate, 0.5% NP-40, pH 6.0
			30. 100 mM HEPES, 50 mM LiCl, 0.1% deoxycholate, pH 7.5
			31. 100 mM Tris, 100 mM KCl, 25% glycerol, 0.1% deoxycholate, pH 7.5
			32. 100 mM potassium acetate, 50 mM NaCl, 0.05% dextran sulfate, 0.1% CHAPS, pH 5.5
			33. 100 mM HEPES, 400 mM NaCl, 0.1 mM EDTA, 0.5 mM DTT, 0.5% NP-40, pH 7.5
			34. 100 mM HEPES, 1 mM EDTA, 0.5 mM DTT, 0.5% NP-40, pH 7.5
			35. 50 mM Tris, 0.5 mM EDTA, 2 mM DTT, 0.5% NP-40, pH 7.5

Figure 2. Solubility of VP22-His in various buffer systems

Following infection of Vero cells with the VP22-His recombinant virus, infected cell pellets were lysed in each of the listed buffers. Soluble cellular components were separated from insoluble components by centrifugation. Anti-VP22 immunoblots were performed to detect the presence of VP22-His in the total lysate (TL), the soluble fraction (Sol), and the insoluble fraction (Insol) of the lysate as described in Materials and Methods.

Figure 3. Purification of VP22 from VP22-His infected cells

6x His-tagged VP22 was purified from VP22-His infected Vero cells as described in Materials and Methods. A) Coomassie-stained SDS-PAGE gel of samples taken during the purification of VP22 from Vero cells infected with the VP22-His recombinant virus. Total lysate = initial lysate of VP22-His infected Vero cells, Soluble = soluble proteins following centrifugation of the total lysate, Flow-through = proteins in the soluble fraction that did not bind the cobalt affinity resin, Elutions 1 and 2 = proteins eluted from the cobalt affinity resin following incubation with elution buffers 1 or 2, respectively. (B) Anti-VP22 immunoblot of samples taken during the purification of VP22 from Vero cells infected with the VP22-His recombinant virus to verify presence of VP22. Samples are as described for (A).