



Expression, Purification, and Antiserum Production of the Truncated UL31 Protein of Herpes Simplex Virus 1

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Background: The UL31 protein of herpes simplex virus 1 (HSV-1) plays an important role in the HSV-1 replication, however, its pinpoint functions in the life cycle of the virus have yet to be adequately elucidated.

Objectives: An antiserum specific for detecting HSV-1 UL31 was prepared as the foundation for future research on the role of UL31 in the course of HSV-1 infection.

Materials and Methods: Recombinant protein of UL31 was expressed in *Escherichia coli*, which was then purified and employed to raise the level of antiserum in mice. Subsequently, western blot and immunofluorescence assay (IFA) were utilized to detect the specific antiserum.

Results: The recombinant UL31 protein consisting of N-terminal 27 aa of UL31 was fused to EYFP and His-tag. It was expressed, purified, and applied to the preparation of the antiserum. Western blot analysis and IFA demonstrated that this antiserum could detect both the recombinant UL31 and the native UL31.

Conclusions: Our results manifest that this antiserum could be conducive to further investigations concerning the roles of UL31 in the HSV-1 infection.

Keywords: *Escherichia coli*; Herpes simplex virus 1; Immune Sera; Recombinant Proteins

1. Background

It is well-known that HSV-1 (herpes simplex virus 1) is a common human virus that can cause a variety of diseases, including mucocutaneous lesions of the mouth, eyes, face, or genitalia, and, seldom, meningitis or encephalitis (1). The UL31 protein contains 306 amino acids (aa), encoded by the HSV-1 *UL31* gene (2-4), and in the life cycle of the virus, UL31 is one of the important structural proteins that has certain characteristics, such as binding with HSV-1 UL34 to form the nuclear egress complex (2-4), altering localization, phosphorylation, and interaction of nuclear lamina components and breaking off the nuclear lamina (5-7), promoting primary envelopment and nuclear exit of nucleocapsids (8-10). Furthermore, UL31 might form a network to allow the anchorage of viral products

for the synthesis and/or packaging of viral DNA into virions (11). Besides, UL31 is involved in both the optimal activation of NF- κ B and the expression of viral gene products (12). However, the precise role of UL31 in the HSV-1 infection remains to be further studied.

2. Objectives

In the current study, a recombinant protein composed of truncated N-terminal 1-27 amino acids (aa) of UL31 fused with His and EYFP tag was expressed in *E. coli* and purified by Ni-NTA affinity chromatography under denatured conditions. Following renaturation, the recombinant protein was employed to raise the level of the antiserum in the BALB/c mice. aa 1-27 of HSV-1 UL31 was chosen for preparing antiserum since this

fragment is the nuclear localization signal (NLS) of UL31 (13) and its antiserum is important for elucidating the function of UL31 NLS in the course of the HSV-1 infection. Subsequently, the results of western blot analysis showed that both the truncated and the native UL31 proteins could be identified by this antiserum. Besides, immunofluorescence assay (IFA) disclosed the subcellular localization pattern of UL31 in cells infected with HSV-1.

3. Materials and Methods

3.1. Plasmids Construct

UL31 ORF (consisting of 918 bp) was amplified by PCR from the genomic DNA of HSV-1 F strain (accession No: KM222725) (14), using KOD-plus-Neo (TOYOBO) with the following primers: UL31-F (5'-CGAAGCTTCGGAATTCATGTATGACACCGACCCCCATC-3') and UL31-R (5'-GCAAGCTTAGGATCCGTCGGCGGAGGAACTCGTCGAA-3'). As performed in our previous efforts (4, 15-20), the purified fragment was treated with *EcoRI* and *BamHI* and ligated into the same treated pEYFP-N1 (Clontech) to get pUL31-EYFP. Then, the DNA fragment encoding N-terminal 27 aa of UL31 was amplified from pUL31-EYFP using primers UL31-F and UL31 (1-27)-R (5'-AAGGATCCAGAGAGGAGCGCGACCGCCGGC-3'). The PCR product was inserted into pEYFP-N1 to produce pUL31 (1-27)-EYFP. Afterwards, the DNA product corresponding to the N-terminal 27 aa of UL31 fused to EYFP was digested with *EcoRI* and *NotI*, and was subcloned into the correspondingly digested pET-28a(+) (Novagen) to construct a recombinant prokaryotic expression plasmid – i.e., pET28a(+)-UL31(1-27)-EYFP. For determining the presence of the target insert in the obtained recombinant plasmid pET28a (+)-UL31 (1-27)-EYFP by PCR, the primer EYFP-R (5'-GAAGATCTCGCTTGTACAGCTCGTCCATGCCG-3') was also applied.

3.2. Expression and Purification of the Recombinant Protein

E. coli BL21 (DE3) cells were transformed with the recombinant plasmid pET28a (+)-UL31 (1-27)-EYFP to express the recombinant protein. Next, the expression and the purification of the recombinant protein were manipulated as done before (20-23). To increase the production of the recombinant protein, the condition of the culture was optimized for expression by varying the temperatures, the concentrations of IPTG, and the duration of induction. Protein expression was assessed by SDS-PAGE. Soluble and insoluble fractions were then analyzed in parallel by 12% SDS-PAGE. The recombinant protein was purified on Ni²⁺-NTA affinity resin following the modified version of the

manufacturer's instructions. The purified recombinant protein was stored at 4 °C for use within 1 week or at –70 °C for future use.

3.3. The Preparation of the Antiserum

The antiserum preparation was carried out as represented previously, except that the recombinant protein was employed in this research (21-23). The antiserum was harvested from the eyeball of the BALB/c mice and stored at –80 °C until further use.

3.4. Western Blot Analysis

Western blot analysis was manipulated as previously reported and we used the antiserum against the recombinant protein (20, 22, 24).

3.5. IFA

To see the subcellular localization of UL31 in cells infected by HSV-1, we infected them with HSV-1 (F) strain with an MOI of 1 at 0h, 12h, and 24h post infection (hpi). Vero cells were subjected to IFA by formaldehyde-based fixation approach, by using the antiserum against the recombinant protein, as mentioned in our previous reports (20, 22). The cell nuclei were counterstained by Hoechst.

4. Results

4.1. The Plasmid Expressing the Truncated UL31 Recombinant Protein was constructed.

In an attempt to produce the specific antibody against HSV-1 UL31 the plasmids pUL31-EYFP and pUL31 (1-27)-EYFP were constructed (data not shown). The DNA fragment encoding UL31 (1-27)-EYFP was digested with *EcoRI* and *NotI* then was subcloned from pUL31 (1-27)-EYFP into the correspondingly digested pET-28a (+) (Novagen) to yield a recombinant prokaryotic expression plasmid pET28a (+)-UL31 (1-27)-EYFP (Fig. 1, lane 3). Afterwards, the recombinant plasmid (pET28a (+)-UL31 (1-27)-EYFP) was examined by PCR (Fig. 1, lane 1 and lane 2) and was subjected to the restriction analysis (Fig. 1, lane 4). The results demonstrate that the plasmid was successfully constructed. In addition, DNA sequencing results show that, in contrast to the HSV-1 F strain, there was no nucleotide mutation in the truncated UL31 protein (data not shown).

4.2. Expression and Purification of the Recombinant Protein

Here we used *E. coli* strain BL21 (DE3) to express the recombinant UL31 protein. The strain has the advantage of lacking the ompT with lon proteases while it harbors the T7 bacteriophage RNA polymerase gene, which makes it possible for the heterologous genes to specifically expressed by the T7 promoter (25, 26). After induction for 4 h at 37 °C with 1.0 mmolL⁻¹ IPTG, although BL21 (DE3) containing the recombinant plasmid did not express under non-

induced condition (Fig. 2A, lane 1), *E. coli* BL21 (DE3) containing the recombinant plasmid expressed in abundance after induction (Fig. 2A, lane 2). An evident band of about 35 kDa, corresponding to the expected M_r of truncated UL31 (1-27)-EYFP-His protein was observed (Fig. 2A, lane 2).

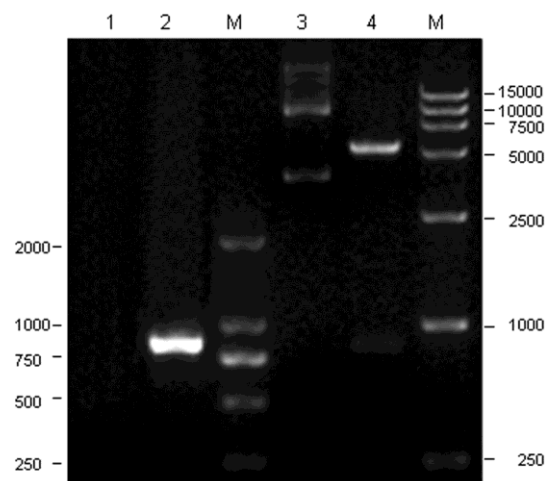


Figure 1. Cloning and verification of the recombinant plasmid pET28a-UL31 (1-27)-EYFP. The negative control (lane 1), PCR product with primers UL31F and EYFP-R (lane 2), the recombinant plasmid (lane 3), restriction enzyme digestion product of the recombinant plasmid with *EcoRI* and *NotI* (lane 4), DNA size markers (lanes M).

Several expression parameters, such as induction IPTG concentrations (Fig. 2A, lane 1-4) and induction time (Fig. 2A, lane 5-8) were manipulated to optimize the expression of the recombinant protein. The recombinant protein exhibited high expression after induction under all the conditions explored in this study. SDS-PAGE analysis indicated that the recombinant protein was largely insoluble in the form of inclusion bodies. Between cell debris pellet (Fig. 2B, lane 3) and the soluble fraction (Fig. 2B, lane 2), the induced recombinant protein was examined primarily in debris pellets (Fig. 2B, lane 3).

To establish an efficient approach to purify the recombinant UL31, an immobilized metal affinity chromatography on Ni^{2+} -NTA resin column, which exhibits a high binding capacity allowing for a rapid and single-step purification, was applied to purify the recombinant protein (27). The successful purification of the purified recombinant protein is evident in SDS-PAGE analysis, since only one clear band corresponding to M_r of about 35 kDa was probed (Fig. 2C, lane 1). After purification, we can acquire about 240 mg of recombinant protein per liter culture. Finally, the BALB/c mice were injected with the purified protein to produce the specific antiserum against this recombinant protein.

4.3. Characterization of the Antiserum against Recombinant Protein

Three immunizations later, the antiserum was sampled from the eyeball of the BALB/c mice, subsequently western blot analysis was carried out to evaluate the specificity and the reactivity of the antiserum. The antiserum could recognize UL31 in the HSV-1 infected Vero cells (Fig. 3, lane 1). As expected, we could not locate a band from the mock-infected Vero cells (Fig. 3, lane 2). Furthermore, western blot analysis demonstrated that the antiserum could detect the full-length UL31 tagged by EYFP protein in the lysates of pUL31-EYFP from the transfected HEK293T cells (Fig. 3, lane 3), whereas no band was observed in the lysates of the untransfected HEK293T cells (Fig. 3, lane 4). These results suggest that the prepared antiserum has an adequate reactivity with the recombinant and also the native UL31.

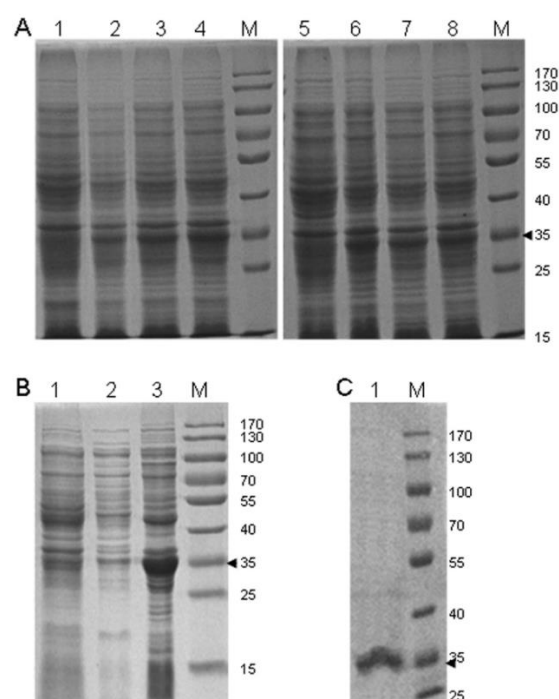


Figure 2. Expression analysis and expression optimization of the recombinant protein. (A) Optimization of the IPTG concentration and the induction duration. Lanes 1 to 4, total protein from the recombinant plasmid transformed BL21 after induction with IPTG: 0, 1.0, 0.2, 0.1 mM, at 37 °C, respectively; lanes 5 to 8, total protein from the recombinant plasmid transformed BL21 after induction with IPTG (1.0 mM) for 0, 4, 8 and 12 h, at 37 °C, respectively. (B) Expression analysis of the recombinant plasmid transformed BL21 non-induced (lane 1) and induced with IPTG separated in supernatant (lane 2) and pellet (lane 3). (C) Purification of the recombinant protein. Lane 1, purified recombinant protein after dialysis. Lanes M, protein markers. Arrowheads indicate the position of the recombinant protein.

4.4. Subcellular Localization of UL31 in HSV-1-Infected Cells

IFA was performed at different times using the prepared antiserum to detect the subcellular localization pattern of UL31 in the HSV-1 infected Vero cells. We fixed and permeabilized the HSV-1-infected Vero cells at 0, 12

and 24 hpi. Next, the cells were blocked with BSA to remove nonspecific binding and allow for reaction with the prepared antiserum. As displayed in **Figure 4**, UL31 predominantly localized near the nuclear rim when cells were infected at 12 and 24 hpi. In contrast, we could not detect specific staining in the HSV-1-infected cells at 0 hpi. These results demonstrate that the prepared antiserum has adequate reactivity and specificity against the native UL31 proteins in the infected cells. Based the results, that UL31 is a nuclear rim-targeted protein.

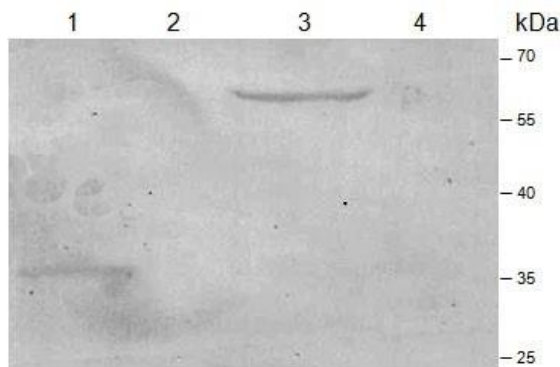


Figure 3. Characterization of the prepared antiserum against the recombinant protein by western blot analysis. Lysates of Vero cells infected with HSV-1 (lane 1) or mock infected (lane 2); lysates of HEK293T cells transfected with pUL31-EYFP (lane 3) or untransfected HEK293T cells (lane 4); the molecular mass of protein marker is labeled.

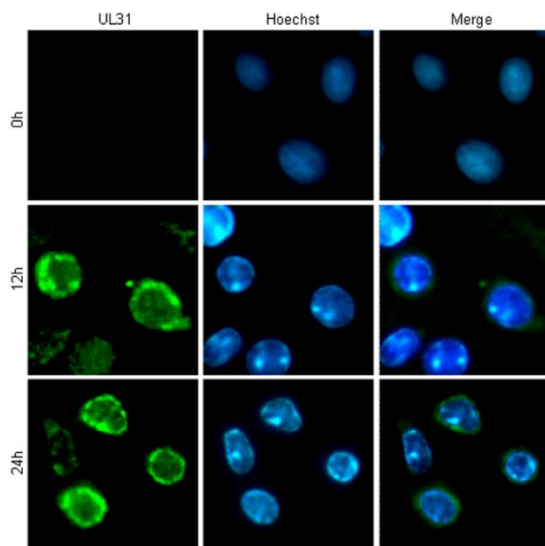


Figure 4. Subcellular localization of UL31 in HSV-1-infected VERO cells. Cells infected with HSV-1 F strain at an MOI of 1. Then IFA was performed at the indicated times post infection with the antiserum against the native UL31 protein.

5. Discussion

Here we produced an antiserum against the truncated UL31 protein that can specifically recognize both the recombinant and the native UL31 proteins. As mentioned before, the precise role of UL31 in the HSV-

1 infection demands further investigation. Consequently, the prepared antiserum is a useful tool for in-depth study of the functions of UL31 or/and recombinant UL31.

It has been reported in that the intracellular localization of different viral proteins may reflect the various functions of the protein and the intracellular localization of viral proteins may also change at different times after infection (28). For example, it has been shown that in Vero cells infected with HSV-1, the localization of HSV-1 UL3 changes from nuclear to small compact nucleosome during the viral replication cycle (29). Besides, HSV-1 UL31 and UL34 have separate but related functions in recruiting appropriate components to nucleocapsid budding sites at the inner nuclear membrane (30). One of the important properties of UL31 is its interaction with HSV-1 UL34, and this interaction is involved in several viral replication processes (2, 3), including (a) co-localization at the nuclear rim (31), (b) changing the localization, interaction, and phosphorylation of nuclear lamina components and disrupting the nuclear lamina (5-7), and (c) facilitating the primary envelopment and nuclear egress of their respective nucleocapsids (8-10). Therefore, it is expected that UL31 primarily displays nuclear rim localization in the HSV-1 infection cells (13, 32). Moreover, both the optimal activation of NF- κ B and the expression of viral gene products involves UL31 (12), therefore it is not unexpected that UL31 is primarily a nuclear-associated protein.

6. Conclusions

In conclusion, we produced an antiserum against the truncated UL31 protein that could specifically recognize both the recombinant and the native UL31 proteins. Consequently, this antiserum could be considered a promising strategy to be used to further our understanding of the UL31 biological functions in the course of the HSV-1 infection.

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Financial Disclosure

The authors declare that they have no competing interests.

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