Acta virologica **54**: 147 – 150, 2010 doi:10:4149/av_2010_02_147

Recombinant N-terminal part of Bovine herpesviru 1 ICP27 protein: its preparation, purification, and use for raising specific antiserum

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Received September 7, 2009; accepted March 24, 2010

Summary. – Recombinant N-terminal part of bovine herpesvirus-1 (BoHV-1) ICP27 protein fused with thioredoxin and His-tag ("the recombinant protein") expressed in *Escherichia coli* was purified by the Ni^{2+}-NTA affinity chromatography and used for the preparation of antiserum by immunization of rabbits. The antiserum recognized the recombinant protein in Western blot analysis and was able to detect BoHV-1 ICP27 in the nucleoli of BoHV-1-infected MDBK cells. These results showed that such an antiserum could serve as a valuable tool in further studies of the functions of BoHV-1 ICP27.

Keywords: Bovine herpesvirus 1, ICP27, recombinant protein, antiserum, immunofluorescence assay

Introduction

BoHV-1 (the subfamily *alphaherpesvirinae*, the genus *Varicellovirus*) is the etiological agent of a number of diseases including infectious bovine rhinotracheitis, infectious pustular vulvovaginitis, infectious balanoposthitis, conjunctivitis, encephalomyelitis, mastitis, abortion, enteritis, and lesions in the interdigital space (Mayfield *et al.*, 1983). The BoHV-1 ICP27 is an early protein consisting of 400 amino acids that may stimulate mRNA 3' processing (Chalifour *et al.*, 1996; Singh *et al.*, 1996). It also specifically transactivates the promoter of glycoprotein C (Hamel and Simard, 2003). Its homolog ICP27 of Herpes simplex virus 1 (HSV-1) is an immediate early protein (Sacks *et al.*, 1985; McCarthy *et al.*, 1989). Amino acid sequence analysis showed only 32% homology between these two proteins, suggesting that they may have different functions. HSV-1 ICP27 has been extensively studied (Sandri-Goldin, 2008), but the functional properties of BoHV-1 ICP27 are not as much understood.

In this work, the antiserum against BoHV-1 ICP27 was raised in rabbits for further characterization of the biological function of BoHV-1 ICP27 during infection. First, we attempted to express a recombinant N-terminal part (100 aa) of BoHV-1 ICP27 fused to thioredoxin and His-tag in *E. coli.* Next, the recombinant protein was purified and used for the preparation of specific antiserum in rabbits. The antiserum was then used for the detection of BoHV-1 ICP27 in virus-infected MDBK cells.

Materials and Methods

Animals and reagents. pET-32a (+) and *E. coli* BL21 (DE3) were purchased from Novagen, all restriction endonucleases and DNA polymerase were from New England Biolabs. The nickel-nitrilotriacetic acid (Ni^{2+}-NTA) affinity resin column was supplied by Roche Diagnostics. Three month-old New Zealand white rabbits used for immunization were from Wuhan Institute of Virology, Chinese Academy of Sciences.

Recombinant plasmid construct. The nucleotides encoding N-terminal 100 amino acids of BoHV-1 ICP27 ORF were amplified from BoHV-1 genomic DNA (Hubei isolate, China) by PCR.

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Abbreviations: BoHV-1 = Bovine herpesvirus 1; HSV-1 = Herpes simplex virus 1; ICP27 = infected cell protein 27; IFA = immunofluorescence assay

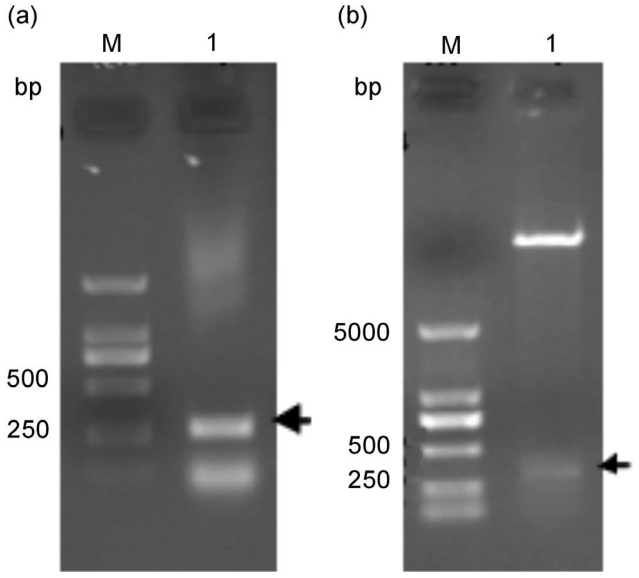

Fig. 1

Construction of the recombinant plasmid

Agarose gel electrophoresis. (a) N-terminal part of BoHV-1 ICP27 gene amplified by PCR (lane 1). (b) recombinant plasmid digested with *Eco*RI and *Bam*HI, the arrow indicates target BoHV-1 ICP27 gene fragment (lane 1). DNA size marker (lanes M).

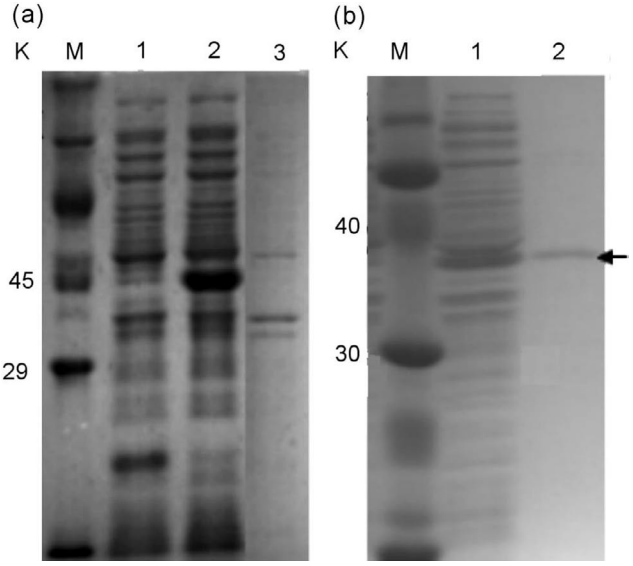

Fig. 2

Expression (a) and purification (b) of the recombinant protein

SDS-PAGE. (a) transformed *E. coli* non-induced (lane 1) and induced with IPTG separated in supernatant (lane 2) and pellet (lane 3). (b) Total bacterial proteins (lane 1) and purified recombinant 32 K protein indicated by arrow (lane 2). Protein size marker (lanes M).

The forward primer (5'-TTGAATTCATGGCGGACCCCGA GATCGC-3') contained an *Eco*RI restriction site (underlined), and the reverse primer (5'-TTTCTCGAGGGCGGGCTGTCT TCGGCG-3') contained an *Xho*I restriction site (underlined). The reactions were performed in a thermocycler: denaturation at 95°C for 30 secs, annealing at 65°C for 30 secs, and extension at 72°C for 30 secs for a total of 30 cycles. The PCR fragment was digested with *Eco*RI and *Xho*I and inserted into pET32a (+) digested with the same enzymes to generate pET32a-ICP27-N100aa, in which ICP27-N100aa was expressed with Trx-Tag™ as fusion protein under the control of T7 promoter. The recombinant plasmid was verified by restriction digestion (Fig. 1) and DNA sequencing (data not shown).

Expression of recombinant protein. E. coli BL21 (DE3) cells were transformed with the recombinant plasmid and the expression of recombinant protein was induced by isopropyl-β-D-thiogalactopyranoside (IPTG) added at the concentration of 1.0 mmol/l, when the culture reached an A_{600} of 0.6. To determine the solubility of the recombinant protein, the induced cells were suspended in PBS and sonicated. The soluble and insoluble fractions were then analyzed in parallel by 12% SDS-PAGE.

Purification of recombinant protein. The cells included in 100 ml culture medium were harvested and resuspended in 30 ml of binding buffer (50 mmol/l Na_3PO_4, 300 mmol/l NaCl, 10 mmol/l imidazole, pH 8.0) containing 1.0 mmol/l phenylmethyl sulfonylfluoride (PMSF) and subjected to sonication. The recombinant protein was purified by immobilized metal-ion affinity chromatography (IMAC) on a nickel-nitrilotriacetic acid (Ni^{2+}-NTA) affinity resin according to the manufacturer's instructions. The purity of the protein was analyzed by 12% SDS-PAGE and the concentration was estimated with an UV spectrophotometer (Bio-Rad).

Antiserum to recombinant protein. Antiserum against the recombinant protein was raised in New Zealand white rabbits. Before immunization, a preimmune serum was collected from each rabbit. The recombinant protein (800 µl, 0.5 mg/ml) was mixed with an equal volume of Freund's complete adjuvant (Sigma) and subcutaneously injected into the footpad and hindquarter of the rabbit. The rabbits were immunized three times with an interval of 14 days and the immune serum was collected from the carotid artery. ELISA assay was used to evaluate antibody titers as described elsewhere (Ryou *et al*., 2003).

Western blot analysis. purified recombinant protein was subjected to 12% SDS-PAGE and electrophoretically transferred to a nitrocellulose membrane. The immune serum against the recombinant protein was used as a primary antibody and alkaline phosphatase (AP)-labeled goat anti-rabbit IgG was used as a secondary antibody (Sigma).

Immunofluorescence assay (IFA). MDBK cells were grown to 80% confluency on glass coverslips and infected with BoHV-1 at the multiplicity of infection of 0.1. the infected cells were fixed with 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 and incubated with the antiserum and FITC-conjugated goat anti-rabbit IgG (Zymed Laboratories). The cells were observed with a Zeiss Axio Observer A1 microscope and images were processed using Adobe Photoshop.

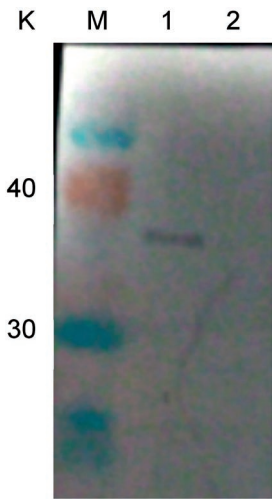

Fig. 3

Western blot analysis of purified recombinant protein

purified recombinant protein detected with the specific antiserum (lane 1), non-transformed *E. coli* lysate as negative control (lane 2).

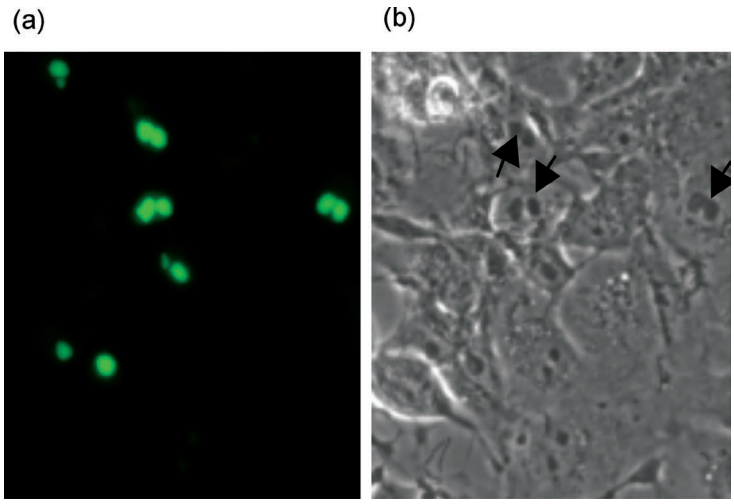

Fig. 4

IFA detection of ICP27 in BoHV-1-infected MDBK cells by specific antiserum

Immunofluorescence photomicrograph (a) and the corresponding phase-contrast photomicrograph (b) are shown. BoHV-1 ICP27 was located predominantly in the nucleolus. Arrows indicated the nucleoli.

Results and Discussion

Expression and purification of the recombinant protein

The recombinant protein was expressed in the *E. coli* BL21 (DE3) strain harboring the recombinant plasmid after induction with IPTG. This bacterium is a preferred host, since it offers several advantages over the most eukaryotic cells such as short generation time, well-established methods for genetic manipulation, and a simple cultivation (Hunt, 2005). SDS-PAGE analysis showed that the expressed recombinant protein could be detected in soluble fraction of induced, but not of non-induced cells (Fig. 2a). SDS-PAGE showed that the relative molecular mass (Mr) of the recombinant protein was 32 K and also, the adequate purity of expressed protein (Fig. 2b).

Production of antiserum specific to the recombinant protein

After the third antigen injection of rabbits, the ELISA assay detected a high titer of antibodies (data not shown). Western blot analysis demonstrated that the prepared antiserum specifically recognized the purified protein (Fig. 3). The sub-cellular localization of BoHV-1 ICP27 during BoHV-1 infection was further examined by IFA using prepared antiserum. IFA of the BoHV-1-infected cells revealed predominant nucleolus localization of BoHV-1 ICP27 (Fig. 4). Based on their size and numbers, these sub-nuclear structures appeared to be nucleoli (Fig. 4). No fluorescence was observed in mock-infected cells or BoHV-1-infected cells probed with the preimmune serum (data not shown). Generally, the localization of a protein determines its function. Localization of BoHV-1 ICP27 in the nucleolus has been proved to be a part of the virus strategy to control both host cell and virus sub-genomic RNA translation. BoHV-1 ICP27 may be involved in 3' processing of mRNA and it might act as a transcription factor (Singh *et al.*, 1996). Thus, its nucleolar localization was expected.

Taken together, the purified recombinant protein and the antiserum prepared against it could be a valuable tool for studying the function of BoHV-1 ICP27 and in the analysis of sub-cellular localization of BoHV-1 ICP27 during infection.

Acknowledgements. We thank Dr. Gareth J. Jones for critical review of the manuscript. This work was supported by the grant 20071010-141 from the Start-up Fund of the Hundred Talents Program of the Chinese Academy of Science, the grant 30870120 from the National Natural Science Foundation of China and the grants 2007003 and 2009007 from the Open Research Fund Program of State Key Laboratory of Virology, P.R. China.

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