Journal of Virological Methods 196 (2014) 56–64

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

High-yield soluble expression of recombinant influenza virus antigens from *Escherichia coli* and their potential uses in diagnosis

Yo Han Jang[a], Seung Hee Cho[a], Ahyun Son[a], Yun Ha Lee[a], Jinhee Lee[a], Kwang-Hee Lee[a], Baik Lin Seong[a,b,*]

[a] Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, South Korea
[b] Translational Research Center for Protein Function Control, Yonsei University, Seoul, South Korea

Article history:
Received 17 April 2013
Received in revised form 16 October 2013
Accepted 22 October 2013
Available online 8 November 2013

Keywords:
Influenza viruses
Recombinant proteins
Bacteria
Antibody library
Fusion partner
ELISA

A B S T R A C T

Although antiviral drugs and vaccines have been successful for mitigating influenza virus infections, the lack of general technical platform for the timely supply of soluble and highly purified influenza viral antigens presents a serious bottleneck for the subsequent analysis for the effective control of the viral disease. Using the *Escherichia coli* (*E. coli*) lysyl tRNA synthetase (LysRS) as a novel fusion partner, this study reports the soluble expression of influenza viral proteins in *E. coli* host, construction of antibody library against the virus, and detection of anti-influenza antibodies using the expressed viral antigens. When influenza A and B viral proteins were fused with the LysRS, the fusion proteins were expressed predominantly as soluble forms and their production yields were high enough to be amenable to immunization protocols in rabbits for antibody generation. The produced antibodies showed high level binding specificity against the respective viral proteins, with cross-reactivity across heterologous viruses within the same type of influenza viruses. In addition, LysRS-HA fusion protein could bind specifically to anti-HA antibodies in the virus-infected mouse serum in widely accepted two detection methods, Western blot and ELISA. These results present a convenient tool for the production of antibodies specific to the virus as well as the rapid detection of influenza viral infections, ultimately contributing to the control of influenza viruses including highly pathogenic H5N1, pandemic H1N1, or the recent H7N9 influenza viruses.

1. Introduction

Influenza virus poses persistently serious threats to human health, which become more pronounced in the event of sporadic pandemics by human-to-human transmission of highly pathogenic viruses (Potter, 2001; Nicholson et al., 2003). While inactivated killed vaccines and cold-adapted live attenuated vaccines can be prepared from cultured influenza viruses, the development of recombinant protein vaccines or in vitro antiviral drug or target screenings need a substantial amount of soluble and highly purified viral antigens. Although *Escherichia coli* systems remain the most preferred choice for the over-expression of heterologous proteins due to distinct advantages including inexpensive carbon source requirements, rapid growth rates, and the simplicity of scale-up (Baneyx, 1999), influenza viral proteins have been hardly amenable to soluble expression in this host, and, in particular, it has been considered extremely difficult to express transmembrane proteins, such as hemagglutinin (HA), neuraminidase (NA), and M2 ion channel protein, as soluble forms.

With maltose-binding protein (MBP), N-utilizing substance-A (NusA), and glutathione S-transferase (GST) at the head of list (Smith and Johnson, 1988; Davis et al., 1999; Fox and Waugh, 2003; Nallamsetty and Waugh, 2006), a number of fusion partners have been advanced as solubility enhancers for the bacterial expression of aggregation-prone proteins (Young et al., 2012). However, most of the fusion partners still failed to support soluble expression for target proteins (Esposito and Chatterjee, 2006), underlining the need for a novel fusion partner that has general versatility in broad-spectrum. Recently, it was reported that RNA-binding proteins (RBP) could serve as novel fusion partners that significantly accelerated the folding and solubility of passenger proteins in *E. coli* (Kim et al., 2007; Choi et al., 2008). Consistent with several reports that RNA had accelerated the folding of interacting proteins (Frankel and Smith, 1998; Rentzeperis et al., 1999; Uversky et al., 2000), interaction with RNA stimulated the folding of the RBPs themselves and also promoted the folding and soluble expression of their fused aggregation-prone proteins (Choi et al., 2008). In addition, the RBP demonstrated superior efficiency in gaining

* Corresponding author at: Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-749, South Korea. Tel.: +82 2 2123 2885; fax: +82 2 362 7265.
E-mail addresses: blseong@yonsei.ac.kr, yhjh0323@yonsei.ac.kr (B.L. Seong).

0166-0934/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jviromet.2013.10.035

and enhancing the solubility to MBP, a widely used fusion partner (Choi et al., 2008), exhibiting a potential to serve as an alternative powerful tool for recombinant protein expression.

With a view to generate antibody library against the influenza viruses, the RBP fusion strategy was applied into the soluble expression and purification of influenza internal and surface membrane proteins by fusion to *E. coli* lysyl tRNA synthetase (LysRS). The prominent ability of the LysRS fusion to induce high yield soluble expression of influenza viral proteins presents significant benefits for the production of viral antigen or antibody libraries for analytical, therapeutic, prophylactic, and diagnostic purposes in numerous non-clinical and clinical investigations.

2. Materials and methods

2.1. Construction of protein expression vectors

pGE-LysRS4 vector constructed in previous work (Choi et al., 2008) was used as a vector for the expression of influenza viral proteins in *E. coli*. The LysRS expression cassette includes LysRS, D6 hexa-aspartic acids linker, TEV recognition site, multicloning site, and the hexa-histidines tag under the T7 promoter (Fig. 1A). The cDNAs each encoding the complete open reading frame (ORF) of individual viral proteins from A/WSN/33 (H1N1), A/Puerto Rico/8/34 (H1N1), and B/Yamagata/16/88 were amplified by reverse transcription PCR (RT-PCR) and then inserted into the pGE-LysRS4 vector.

2.2. Protein expression

Protein expression and SDS-PAGE analysis were performed as described previously (Choi et al., 2008). Each expression plasmid was transformed into the *E. coli* expression host, BL21 (DE3) pLysS. A single colony of transformants was inoculated into 3 ml of LB containing both 50 μg/ml ampicillin and 34 μg/ml chloramphenicol. The culture medium was then diluted into 25 ml of the fresh LB containing the same antibiotics and the cells were cultured to the optical density (OD) of 0.5 at 600 nm. Expression of proteins was induced for 6 h at 20–30 °C by the addition of 1 mM IPTG. After centrifugation, the cell pellets were suspended in 0.3 ml of PBS and disrupted by sonication. The cell lysates were centrifuged at 11,000 × *g* for 12 min and separated into soluble and pellet fractions. Each fraction was then mixed with the same volume of 2× SDS loading buffer and boiled. These samples were subjected to SDS-PAGE and visualized by staining with Coomassie brilliant blue R-250.

2.3. Purification and quantification of fusion proteins

Proteins were purified from 1 l culture of each transformant using nickel affinity chromatography. The harvested cell pellets were suspended in 5 ml of equilibration buffer A (20 mM Tris–HCl (pH 7.5), 300 mM NaCl, 10% glycerol, 2 mM 2-mercaptoethanol, and 5 mM imidazole) and disrupted by sonication. The soluble fractions were recovered by centrifugation at 11,000 × *g* for 20 min and then applied onto HiTrap chelating HP column (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, UK). After washing, proteins were eluted with 50 ml of elution buffer with the same composition of equilibration buffer as above except for the linear gradients of imidazole ranging from 10 mM to 300 mM. The fractions containing proteins of interest were pooled, concentrated, dialyzed against the buffer containing 50 mM Tris–HCl (pH 8.0), 50 mM NaCl, 1 mM EDTA, and 1 mM DTT in 50% glycerol. After SDS-PAGE, the purified proteins were quantified by gel densitometry scanning of the Coomassie-blue stained protein band using bovine serum albumin (BSA) (Amresco, Solon, OH, USA) of a known concentration as control.

2.4. Animal cells and influenza viruses

Madin–Darby canine kidney (MDCK) cells were maintained in minimal essential medium supplemented with 10% fetal bovine serum. To evaluate the binding specificities of antisera collected from the rabbits immunized with the fusion proteins, MDCK cells were infected with 1 multiplicity of infection (MOI) of each of four influenza A viruses, A/WSN/33 (H1N1), A/Singapore/6/86 (H1N1), A/Sydney/5/97 (H3N2), A/Puerto Rico/8/34 (H1N1), and of three influenza B viruses; B/Lee/40, B/Yamagata/16/88, and cold-adapted B/Lee/40 (Seo et al., 2008), and the cells were harvested 24 h after the infections for Western blots.

2.5. Immunization and polyclonal antibodies production in rabbits

Rabbit experiments were conducted in the animal facilities of the LabFrontier (Anyang, South Korea). To obtain polyclonal antibodies specific to influenza viral proteins, two rabbits were immunized with each of purified LysRS-fused recombinant influenza viral proteins. Each 0.5 mg of LysRS-fused protein emulsified with Freund's complete adjuvant was injected intradermally at multiple sites on the back of each rabbit. Starting from four weeks after the first immunization, each animal was boosted twice by an additional 0.2 mg of the protein and adjuvant mixtures with the interval of two weeks. Sera samples were collected at seven days after each boosting and the final sera samples were aliquoted and stored at −80 °C. The experimental protocol was reviewed and approved by Institutional Animal Care and Use Committee (IACUC) of LabFrontier.

2.6. Mice infection and serum preparation

6-Week-old female BALB/c mice were infected intranasaly with 10^6 PFU of attenuated mutant strain of A/Puerto Rico/8/34 (H1N1) virus (Jang et al., 2013) or mock-infected with PBS as a control. Three weeks later the mice blood samples were collected from the mice via retro-orbital bleeding and clotted at 4 °C overnight to collect sera samples. Mouse study was carried out in strict accordance with the recommendations of the Korean Food and Drug Administration (KFDA) guidelines. Protocols were reviewed and approved by the IACUC of the Yonsei Laboratory Animal Research Center (YLARC) (Permit number: 2012-0094).

2.7. ELISA

Microtiter 96-well plates were coated with 100 ng/well of LysRS-fused influenza viral protein and incubated at 4 °C overnight. The plates were washed and blocked by 1% BSA for 1 h at room temperature (RT). 100 μl of sera with various dilutions were added to the wells for 1 h, and the same volume of a secondary goat anti-rabbit IgG antibody or goat-anti mouse IgG antibody conjugated with HRP (Sigma–Aldrich, St. Louis, MO, USA) was treated for 1 h. After washings, substrate solution was added to the well and the plate was incubated in the dark. The colorimetric reaction was stopped by the addition of 100 μl/well of 1 M H_2SO_4 and the absorbance was read at 492 nm on ELISA reader (FLUOstar OPTIMA, BMG LABTECH, Offenburg, Germany).

2.8. Western blot analysis

MDCK cells infected with one MOI of each influenza virus were subjected to Western blot analysis using each final serum