JJBS

Volume 15, Number 3,September 2022
ISSN 1995-6673
Pages 361 – 364
https://doi.org/10.54319/jjbs/150303

Jordan Journal of Biological Sciences

Prokaryotic Expression of Murine Cellular Prion Protein for *In vitro* Evaluation

Hoang-Anh Le-Dao[1,2,3,+], Yen-Vy Le-Nguyen[1,2,3,+], Hieu Tran-Van[1,2,3,*]

[1]Department of Molecular and Environmental Biotechnology, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City 700000, Vietnam;[2]Laboratory of Biosensors, Faculty of Biology and Biotechnology, University of Science, Ho Chi Minh City 700000, Vietnam;[3]Vietnam National University, Ho Chi Minh City 700000, Vietnam

Received: June 4, 2021; Revised: August 2, 2021; Accepted: August 25, 2021

Abstract

M cell (Microfold cell) targeting is a potential strategy for oral vaccine development due to its role in presenting antigen to mucosal lymphoid tissue. Numerous researches have focused on directing vaccine antigens to M cell receptors, based on their affinity with specific ligands. This study was aimed to biosynthesize murine cellular prion protein (mPrP^C), a highly expressed receptor on the M cell apical surface. The mPrP^C coding sequence was obtained by PCR for construction of pGEX-5X-1-*mprpc* plasmid. Then, recombinant plasmid was induced for expression in *E. coli* BL21(DE3) cells by IPTG. The overexpression of mPrP^C was confirmed by SDS-PAGE and Western blotting. Since the expressed protein was found to remain mostly in insoluble fraction, DTT and glycerol were used to improve the solubility. This soluble, recombinant mPrP^C could be used for *in vitro* evaluation, and also on further research for developing antigen targeting M cell.

Keywords: cellular prion protein, gene expression, M cells, microfold cells, SDS-PAGE.

1. Introduction

Gastrointestinal mucus is the largest surface of the human body frequently exposed to microbial pathogens from the environment. The most effective treatment for the infection is antibiotic which can cause antibiotic-resistance (Ventola, 2015). This is a serious global concern that requires an alternative solution for these pathogens. Oral vaccine protects against mucosal infection because of its efficiency in inducing mucosal immune responses, inclusively elicit antigen-specific secretory IgA, or even inducing systemic immune responses (Baumann, 2008). However, the number of oral vaccines is still limited due to some disadvantages. For example, vaccine antigens resist the harsh conditions, large-area surface in the gastrointestinal tract resulting in antigen dispersion; moreover, the intestine tends to become immune-tolerance because of continuously being exposed to numerous external antigens (Pabst and Mowat, 2012). To overcome these challenges, multiple strategies for targeting vaccines to gut-associated lymphoid tissue (GALT), where the antigen sampling takes place and immune responses induced, have been implemented. A specialized cell that has an important role in antigen sampling is M cell (Microfold cell), which is found overlying Peyer's patches (PP) and containing lymphoid follicles of GALT (Sansonetti and Phalipon, 1999). Current knowledge on M cell has shown that antigen uptake occurs via surface receptors, and antigen transports through dorm structure to beneath lymphoid tissue and present to PP's immunocytes, leading to the production of specific IgA (Neutra *et al.*, 2001, Azizi *et al.*, 2010). Because of these characteristics, orientating vaccine antigen to M cell receptors is a promising strategy and has been carried out in many studies (Huynh *et al.*, 2019, Shima *et al.*, 2014). For research on this strategy, it is necessary to identify the ligands of M cell receptors and verify the interaction between them. PrP^C is a highly expressed receptor on M cell apical membrane and proved to have interactions with Heat shock protein (Hsp)60 from *Brucella abortus* (Nakato *et al.*, 2012), which is found to be an immunodominant antigen of many pathogenic bacteria (Kaufmann, 1990). These features make PrP^C-Hsp60 a potential receptor-ligand for M cell targeting vaccine antigen.

In this present study, we proceeded on expressing the recombinant murine PrPC receptor fused with GST-tag in E. coli prokaryotic expression system in which GST tag would enhance solubility and support for in vitro interaction evaluation with recombinant Hsp60 ligand in further study; moreover, this result was a prerequisite for studies on the application of this receptor-ligand in oral vaccine development.

2. Materials and Methods

Isolation of *mprpc* gene: *mprpc* gene corresponding to 1792-2415 of *Mus musculus* prion protein (Prnp) mRNA (NM_001278256.1) was amplified by PCR using a DNA

[+] Co-first authorship
* Corresponding author. e-mail: tvhieu@hcmus.edu.vn.

 © 2022 Jordan Journal of Biological Sciences. All rights reserved - Volume 15, Number 3

Engine Tetrad thermal cycler (MJ Research, USA) with forward primer (5'-ggatccccaaaaagcggccaaagcctgg-3') containing a *Bam*HI restriction site underlined, and reverse primer (5'-ctcgagctaggatcttctcccgtcgtaatag-3') containing a *Xho*I restriction site underlined. The PCR reaction (50 µl total volume) contains 2x MyTaq Red Mix (Bioline, Canada), 25 µl; 15 µM primer (PhuSa Biochem, Vietnam), 1 µl each; 200 ng template plasmid pET-*mprpc* from our previous work (Truong-Ha *et al.*, 2019), 1 µl; and sterile distilled water, 22 µl. PCR conditions were performed: initial denaturation 2 min at 95ºC, followed by 30 cycles of 15 sec at 95ºC, annealing for 15 sec at 65ºC, extension for 15 sec at 72ºC, and final extension at 72ºC for 10 min before cooling to 30ºC.

2.1. Recombinant plasmid construction:

mprpc gene and cloning vector pGEX-5X-1 containing GST were treated with the same restriction enzymes *BamH*I and *Xho*I (Thermoscientific, USA) to produce compatible sticky ends. Subsequently, the treated gene was ligated to digested plasmid using T4 DNA ligase (Thermoscientific, USA).

2.2. Cloning procedure

the competent *E. coli* DH5α cells (New England Biolabs, USA) was prepared by ice-cold $CaCl_2$ 100 mM buffers using the Hanahan Method (Green and Sambrook, 2018). The ligated product was transformed into *E. coli* DH5α by incubating the mixture of competent cells and ligated product on ice for 10 min, then placing at 45°C for 90 sec, and cooling down on ice for 10 min (heat-shock method) (Sambrook and Russell, 2001). The transformed cells were screened onto Luria-Bertani (LB) agar plate containing ampicillin (LB-Amp) for selection of recombinant clones. Transformants grown on plate were screened by colony PCR using the same thermal cycler and condition, which was mentioned in isolation of *mprpc*. PCR product was showed on 1.5% agarose gel. The sequence of inserted gene from positive colonies was confirmed by sequencing alignment with the obtained sequence gene. Successfully constructed recombinant plasmid pGEX-5X-1-*mprpc* was purified using EZ-10 Spin Column Plasmid DNA Minipreps Kit (Biobasic, Canada). Purified plasmid was transformed into *E. coli* BL21(DE3) (New England Biolabs, USA) chemically competent cells for overexpression of recombinant protein. The colonies grown on LB-Amp agar plate were confirmed for carrying recombinant plasmid by colony PCR using same setting mentioned above with pGEX5 primer (5'-ggcaagccacgtttggtg-3') and pGEX3 primer (5'-ggagctgcatgtgtcagagg-3').

Protein expression and Western blotting: the positive *E. coli* BL21(DE3) colony was cultivated in LB broth supplemented with ampicillin (100 ug/ml) overnight at 37°C. On the following day, a subculture was made by transferring the inoculum into fresh LB-Amp with a ratio of 1:10 (v/v), and it was then incubated in shaker until OD_{600} reached 0.6-0.8. The expression was induced by IPTG at final concentration of 0.1 mmol/L at 16°C for 16 h in a 200-rpm shaker incubator. The culture was centrifuged to obtain cell pellet, which was dissolved in lysis buffer, PBS which composed of 137 mM of NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, pH 7.4. To increase soluble fraction, DTT (2 mM) and glycerol (10%) were added to cell lysis buffer. Suspended cells were lysed by sonication (15 cycles, pulse for 10 sec on and 10 sec off) and then were centrifuged at 10,000 rpm for 10 min to separate soluble and insoluble fractions. The protein expression analysis of recombinant $mPrP^C$-GST was conducted simultaneously with the GST protein expressed from cloning plasmid pGEX-5X-1 with the same procedure. Total cellular protein, soluble and insoluble protein phase was subjected to a SDS-polyacrylamide gel electrophoresis (SDS-PAGE method) according to standard protocol (Sambrook and Russell, 2006) and Coomassie Brilliant Blue staining. Protein separated after SDS-PAGE was then transferred onto a nitrocellulose membrane via Trans-Blot SD Semi-Dry Transfer Cell (Biorad, USA). The membrane was blocked with skim milk 5% in PBS + Tween20 (0.05%) (PBS-T). Blocked membrane was incubated with antibody (Anti-GST monoclonal mouse HRP (Proteintech, USA)) (1:50 000) for 30 mins. The protein bands were visualized by adding TMB substrate (Thermoscientific, USA).

3. Results

The pGEX-5X-1-*mprpc* plasmid construction was described in Fig. 1A. The insertion of *mprpc* was identified by nucleotide sequencing on positive clone to confirm that the cloned gene was matched with designed sequence and correct direction.

The recombinant plasmid was isolated and transformed to *E. coli* BL21(DE3) expression host and recombinant clones were screened by colony PCR. The difference in fragment length in Fig. 1B, lane 2-4 after PCR using pGEX5/pGEX3 primers identified the positive clone containing pGEX-5X-1-*mprpc* recombinant plasmid.

$mPrP^C$-GST expression was induced with IPTG at low temperature. The expression was analyzed by SDS-PAGE, simultaneously with the expression of GST protein in the same condition. The result showed that the expression of $mPrP^C$ fused GST-tag was seen as a band at approximately 49 kDa size, corresponding with the sum of the 23 kDa $mPrP^C$ protein and the 26 kDa GST-tag (Fig. 2A, lane 4), and this band was not seen in total cellular protein of controls (Fig. 2A, lane 1-3). When analyzing soluble and insoluble fractions of induced cell lysate, the $mPrP^C$-GST protein band was seen mostly in insoluble fraction (Fig 2A, lane 5, 6). After adding DTT 2 mM and glycerol 10% to cell lysis buffer, the solubility of expressed protein was improved by about 147.8% (analyzed using ImageJ software) and could be clearly observed in Fig. 2A, lane 8. In addition, the expression of target protein was confirmed by Western blot using specific anti-GST antibody. Figure 2B showed that the antibody bound to GST-fused protein. These results confirmed that the $mPrP^C$ was expressed successfully and can be obtained from soluble fraction of cell lysate in the presence of DTT and glycerol.

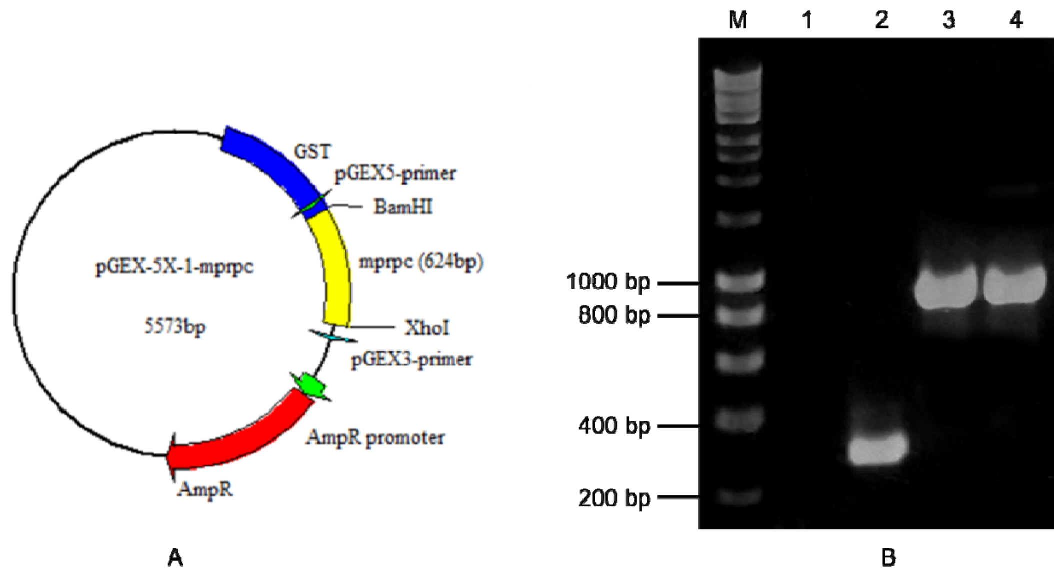

Figure 1. Construction of E. coli BL21(DE3) harboring pGEX-5X-1-mprpc. A: recombinant vector map pGEX-5X-1-mprpc. B: Bacterial colony screening by PCR. M: DNA HyperLadder, lane 1: negative control, lane 2: colony PCR with pGEX-5X-1 transformant, lane 3-4: colony PCR with pGEX-5X-1-mprpc transformants.

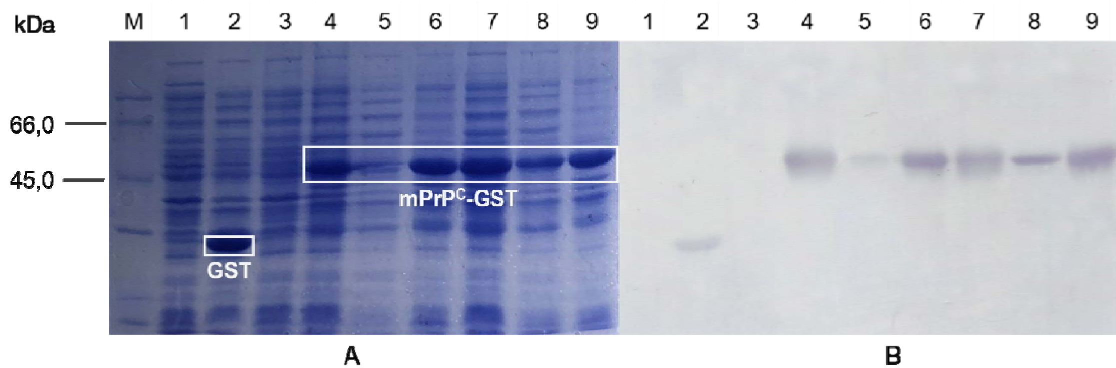

Figure 2. SDS-PAGE (A) and Western blotting (B) analysis of mPrP^C-GST expression. M: protein molecular weight standard, lane 1: E. coli BL21(DE3) cell lysate, lane 2: total cellular protein of induced E. coli BL21(DE3)/pGEX-5X-1, lane 3: non-induced E.coli BL21(DE3)/pGEX-5X-1-mprpc, lane 4-6: total cellular protein, soluble fraction, insoluble fraction of clone expressing mPrP^C-GST, lane 7-9: total cellular protein, soluble fraction, insoluble fraction of clone expressing mPrP^C-GST with DTT and glycerol supplemented in lysis buffer.

4. Discussion

M cell is a promising target in the development of oral vaccine, an effective prevention method for mucosal infectious diseases (Wang *et al.*, 2014, Kim *et al.*, 2012). Due to the important role of M cell in antigen entry to the mucosal immune system, we have been currently focusing on M cell targeting strategies. Many studies have shown that PrP^C membrane receptor of M cell has an affinity for Hsp60 protein from *Brucella abortus* (Edenhofer *et al.*, 1996, Nakato *et al.*, 2012). Therefore, we conducted to express recombinant murine PrP^C (mPrP^C) for being used as a source for *in vitro* interaction evaluation with recombinant Hsp60. The result of this study was a prerequisite for further studies.

Expressed mPrP^C-GST was aggregated in insoluble fraction of cell lysate. This was a disadvantage for the next steps of interaction evaluation. We considered using several chemical agents to improve soluble protein yield. DTT and glycerol were seen to have the most effectiveness leading to a deductive explanation as follows: mPrP^C-GST was expressed and formed non-classical inclusion bodies containing insoluble proteins encapsulated correctly folded proteins (Peternel *et al.*, 2006), in *E. coli* cytoplasm. The presence of glycerol in lysis buffer inhibited protein aggregation and DTT has the ability to break disulfide bonds between surrounding precipitated, misfolded protein, helped release proper folded soluble protein.

As many other specific proteins from animal organisms, isolation of PrP^C from gut is a time-consuming and intensive laboratory procedure (Lee, 2017, Pan *et al.*, 1993). Hence, attempts of overexpressing high-yield PrP^C were described. In Weiss *et al.* work (Weiss *et al.*, 1995), cellular prion protein from Syrian golden hamster fusion GST was synthesized using three different systems including *E. coli*. To compare with solubilizing method of this study, DTT and glycerol were used as described below, while recombinantly expressed Syrian golden hamster PrP^C was soluble only in the presence of 2%

sarcosyl since it has the ability to encapsulate proteins and disrupt aggregates (Tao *et al.*, 2010). As mentioned, we used several chemical agents to solubilize fusion protein, and it was found that the solubilizing effect of sarcosyl was lower than that of DTT and glycerol combination (data not shown), which led us to use these reagents for protein solubility improvement.

5. Conclusion

In summary, the soluble form of murine cellular prion protein was successfully biosynthesized and could play a vital role as a potential receptor of M cell for targeting oral vaccine development. *In vitro* interaction evaluating with Hsp60 ligand, *ex vivo* and *in vivo* evaluation could be performed as a premise for further researches on Hsp60 ligand application as a vaccine orientating tag.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

Authors declare that there is no conflict of interest.

References

Azizi A *et al.* 2010. Enhancing oral vaccine potency by targeting intestinal M cells. *PLoS Pathog.,* **6(11):** e1001147.

Baumann U. 2008. Mucosal vaccination against bacterial respiratory infections. *Expert Rev Vaccines.,* **7(8):** 1257-1276.

Edenhofer F *et al.* 1996. Prion protein PrPc interacts with molecular chaperones of the Hsp60 family. *Journal of virology.,* **70(7):** 4724-4728.

Green MR and Sambrook J. 2018. The Hanahan Method for Preparation and Transformation of Competent Escherichia coli: High-Efficiency Transformation. *Cold Spring Harb Protoc,* **2018(3)**.

Huynh K-Q *et al.* 2019. Cloning, expression, purification and interaction evaluation of 30 amino acids in C terminus of Clostridium perfringens toxin with its receptor Claudin-4. *Science and Technology Development Journal - Natural Sciences.,* **2:** 47-55.

Kaufmann SH. 1990. Heat shock proteins and the immune response. *Immunol Today.,* **11(4):** 129-136.

Kim S-H, Lee K-Y and Jang Y-S. 2012. Mucosal Immune System and M Cell-targeting Strategies for Oral Mucosal Vaccination. *Immune network.,* **12(5):** 165-175.

Lee CH. 2017. A Simple Outline of Methods for Protein Isolation and Purification. *Endocrinology and metabolism (Seoul, Korea).* **32(1):** 18-22.

Nakato G *et al.* 2012. Cutting Edge: Brucella abortus exploits a cellular prion protein on intestinal M cells as an invasive receptor. *J Immunol.,* **189(4):** 1540-1544.

Neutra MR, Mantis NJ and Kraehenbuhl JP. 2001. Collaboration of epithelial cells with organized mucosal lymphoid tissues. *Nat Immunol.,* **2(11):** 1004-1009.

Pabst O and Mowat AM. 2012. Oral tolerance to food protein. *Mucosal Immunol.,* **5(3):** 232-239.

Pan KM *et al.* 1993. Conversion of alpha-helices into beta-sheets features in the formation of the scrapie prion proteins. *Proceedings of the National Academy of Sciences of the United States of America.,* **90(23):** 10962-10966.

Peternel Š *et al.* 2006. Nonclassical inclusion bodies in *Escherichia coli*. *Microbial Cell Factories.,* **5(1):** P23.

Sambrook J and Russell DW. 2006. SDS-Polyacrylamide Gel Electrophoresis of Proteins. *CSH protocols.,* **2006**.

Sambrook J and Russell DW. 2001. **Molecular cloning: a laboratory manual**. Cold Spring Harbor Laboratory Press, New York.

Sansonetti PJ and Phalipon A. 1999. M cells as ports of entry for enteroinvasive pathogens: mechanisms of interaction, consequences for the disease process. *Semin Immunol.,* **11(3):** 193-203.

Shima H *et al.* 2014. A novel mucosal vaccine targeting Peyer's patch M cells induces protective antigen-specific IgA responses. *Int Immunol.,* **26(11):** 619-625.

Tao H *et al.* 2010. Purifying natively folded proteins from inclusion bodies using sarkosyl, Triton X-100, and CHAPS. *Biotechniques.,* **48(1):** 61-64.

Truong-Ha M-N, Huynh K-Q and Tran-Van H. 2019. Cloning, expression, and refolding prion protein (PrPC) from murine fused with GST-tag. *Can Tho University Journal of Science.,* **55:** 16-22.

Ventola CL. 2015. The antibiotic resistance crisis: part 1: causes and threats. *Pharmacy and Therapeutics.,* **40(4):** 277-283.

Wang M *et al.* 2014. Roles of M cells in infection and mucosal vaccines. *Human vaccines & immunotherapeutics.,* **10(12):** 3544-3551.

Weiss S *et al.* 1995. Overexpression of active Syrian golden hamster prion protein PrPc as a glutathione S-transferase fusion in heterologous systems. *J Virol.,* **69(8):** 4776-4783.