



Original Research Article

Expression, Extraction and Purification of Respiratory syncytial Virus Matrix Protein from Transformed *E.coli* (BL21)

Layla F. Ali^{1*}, Raghad G.Al-Suhail¹ and Faisal G.Nasir²

¹Department of Biology, College of Science, University of Baghdad/Iraq

²Virology Department, Central Public Health, Laboratory /Ministry of Health/ Iraq

*Corresponding author

A B S T R A C T

Keywords

Matrix protein, cloning, expression, purification, transformation.

In this study, The Matrix protein of Respiratory Syncytial Virus, the viral member of the *Pneumovirinae* (family *Paramyxoviridae*, order *Mononegavirales*), has been inserted in pET16b Plasmid was introduced to a competent *E. coli* (BL21) by heat shock. *E.coli* which was successfully been transformed, was cultured in Luria broth (+ampicillin) and the expression of RSV M Protein was induced by adding 1M (PTIG). RSV M protein was extracted from transformed *E.coli* and purified by Affinity Chromatography using Ni-NTA Agarose beads. The protein was separated by SDS PAGE gel electrophoresis and identified according to its molecular weight which is approximately 30 kDa.

Introduction

Human respiratory syncytial virus (HRSV), a paramyxovirus of the genus *Pneumovirus*, is the main causative agent of pneumonia in infants. In a recent laboratory study, it was among the four most frequently confirmed causes of pneumonia in adults, together with *Streptococcus pneumoniae*, influenza virus and *Mycoplasma pneumoniae* (Zambon, et al 2001). Studies suggest that vaccines formed from attenuated viruses, humanized neutralizing monoclonal antibodies and specific antiviral compounds are the most suitable tools for controlling HRSV infection (Teresita, et al., 2013).

The determination of viral protein function at the molecular level during the virus growth cycle may be the first step towards

improving these reagents. Virions of HRSV are polymorphic, with a membranous envelope. The viral glycoproteins G, F and SH are embedded in the outer part of the membrane and the inner surface contacts the matrix (M) protein. By analogy with other paramyxoviruses, it is thought that homologous interactions of M protein molecules form a sheet that interacts with the viral ribonucleoprotein (vRNP) and with the cytoplasmic tails of the viral glycoproteins. The interaction of the M protein with the vRNP, which is composed of the viral N protein tightly bound to the vRNA or cRNA to form the nucleocapsids, and the L protein bound to its cofactors, the phosphoproteins P and M2-1, is mediated by the N protein (Ghildyal, et al., 2002).

The M protein is crucial in extracellular virus particle formation (Teng and Collins, 1998). It results in the formation of extracellular infectious virus or infectious filamentous structures that protrude from infected cell membranes, in the absence of the ecto- and transmembrane domains of the SH, G and F glycoproteins, but in the presence of the F cytoplasmic tail (5-Oomens, et al., 2003). When expressed alone, M protein is able to bind cellular plasma membranes. This binding is stabilized by surface expression of the viral glycoproteins, and its co-localization with the F protein (Henderson, et al., 2002).

The M proteins of several enveloped viruses, when expressed in the absence of the remaining viral proteins, promote the formation of membranous vesicles within which the M protein is released to the extracellular medium (Burke, et al., 1998). The co-expression of viral glycoproteins or nucleocapsids increases the formation of these virus-like particles (VLPs) (Ulloa, et al., 1998). This suggests that the M protein may promote paramyxovirus budding by acting as a bridge between the nucleocapsid and the viral envelope. It was suggested that the M protein binds simultaneously to cellular actin and the viral RNPs, facilitating transport of these viral structures to the plasma membrane location into which the mature viral glycoproteins are inserted (Wakefield and Brownlee, 1989).

This study was aimed to cloning the Matrix protein of HRSV in *E.coli* as safe host for production of this protein for therapeutical applications.

Materials and Methods

E.coli BL21 (DE3) was used in this study as maintenance and expression host. Genomic RNA was isolated from RSV infected Vero cells using CsCl density gradients to isolate

the ribonucleoprotein nucleocapsid. Treatment with Guandine HCl releasing the genomic RNA which was RT using random hexameric primers. The M ORF was subsequently amplified using primers containing a BamHI restriction site that allows in-frame cloning with the His tag and factor Xa cleavage site in pET 16b (Novagen). The amplified PCR fragment was gene cleaned, restricted with BamHI and ligated into BamHI digested, CIAP treated, pET 16b vector using T4 ligase.

The ligation mix was used to transform competent *E. coli* by using heat shock at 42°C for 3 minutes, plated onto LB-agar (29g dissolved in 1L of distilled water, the pH was adjusted to 7) +ampicillin (100µg/ml) and incubated overnight at 37°C. Transformed bacteria were cultured in LB (+ampicillin) and incubated in shaker incubator at 22°C for 18 hours. Expressed protein was extracted and purified.

Expression, Extraction and purification of RSV M protein

Transformed bacteria were cultured on LB agar+ampicillin. After incubation 18 hours at 37°C, one colony was picked and inoculated into 100 ml LB broth and was incubated in a shaker incubator for 18 hours at 22°C. One ml of overnight culture broth was added to each one of six flasks containing one liter of 2 xYT broth with +ampicillin. The six flasks were incubated in shaker incubator at 22°C, after 5 hours the expression of RSV M protein was induced by adding 1 ml of 1M PTIG (isopropanol β-D-1thiogalagtopyranoside), the incubation was continued to 18 hours. The bacterial culture was transferred into special falcon tubes, centrifuged at 4500 rpm for 15 Minutes at 4 degree centigrade. The pellet was resuspend in Tris buffer containing 50 mM Tris, 5mM MgCl₂, 5 mM CaCl₂ and 200mM NaCl, PH:7.2. The suspension was

subjected to sonication at 4×10^4 circle for 2.5 minutes. The detergent CHAPS 2.5ml was added to the suspension, protease inhibitor, DNAase and RNAase were added. cell lysate was centrifuged at 2500 rpm for 30 minutes at 11°C. supernatant was kept (contain protein). The supernatant was added gently on 4 ml of equilibrated QIAGEN Ni-NTA Agarose beads solution (50%) and the tube was filled with Tris buffer and was sealed with parafilm. The tube was put in a rotor in cold room for one hour (4°C). The mixed was spanned down (1000 rpm) and the supernatant was removed. The beads were suspended In 10 ml phosphate buffer + 50 mm Imadazole RT, then the suspension was loaded into the column. The supernatant was left to flow through and stopped before the agarose dries. The protein was eluted with deferent concentration of imidazole (100, 150, 200, 250, 300, 500) mM. Eluations were collected in separated tubes. The eluted material was centrifuged in the J2-21 at 12,000 rpm for 15 minutes. The aliquots were saved for loading on SDS-PAGE gel.

Loading protein elution on SDS PAGE gel

A volume of 15µl of each elute was added to separate appendorff tubes, 5µl of loading buffer was added to each one, incubated in 95°C for 5 minutes. The protein samples were loaded in wells in SDS PAGE gel for gel electrophoresis and run at 200 V for 45 minutes. The gel was stained with Comassei Brilliant Blue dye for 1 hour then destained. The bands of proteins was observed, the molecular weight of the protein was determined by comparing to the reference protein ladder (BioLabs 10-250 kDa).

Results and Discussion

The Plasmid PET16b was successfully transformed into *E.Coli* (BL21) By using

heat shock as there were colonies appeared on Luria agar plate containing ampicillin (Figure-1). Heat shock in presence of Ca^{+2} cause a significant increase in the electrical conductivity and permeability of the cell plasma membrane. It is usually used in molecular biology as a way of introducing some substance into a cell, such as loading it with a molecular probe, a drug that can change the cell's function, or a piece of coding DNA.. In molecular biology, the process is often used for the transformation of bacteria (Neumann, et al., 1982).

M protein of HRSV was produced by expression host *E.coli* BL21 using Induction by IPTG as the extracted protein gave O.D. of 6.309 at 280nm by Nanodrop after purification by affinity chromatography. The purified protein gave a clear band of 30 kDa band was identified against a high molecular weight protein ladder (Figure-2). The pET 16b System is the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*. Target genes are cloned in pET plasmids under control of strong bacteriophage T7 transcription.

Expression is induced by providing a source of T7 RNA polymerase in the host cell. T7 RNA polymerase is so selective and active that almost all of the cell's resources are converted to target gene expression; the desired product can comprise more than 50% of the total cell protein a few hours after induction. Another important benefit of this system is its ability to maintain target genes transcriptionally silent in the uninduced state. The expression is promote under *lacUV5* control, and expression is induced by the addition of IPTG (Hochuli, et al., 1982). The DNA sequence specifying a string of six to nine histidine residues is frequently used in vectors for production of recombinant proteins.

Figure.1 Growth of transformed *E. Coli* (BL21) on Luria agar +ampicillin

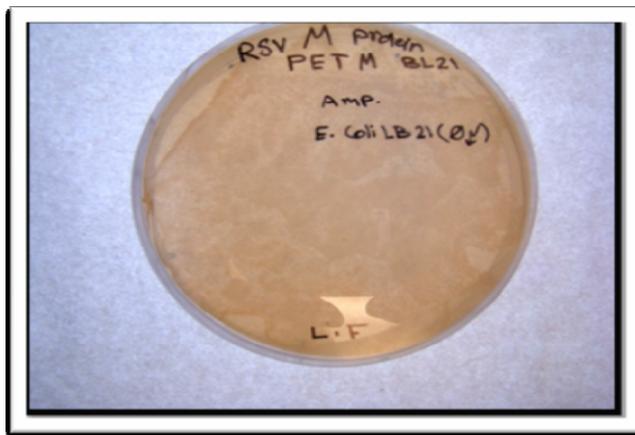
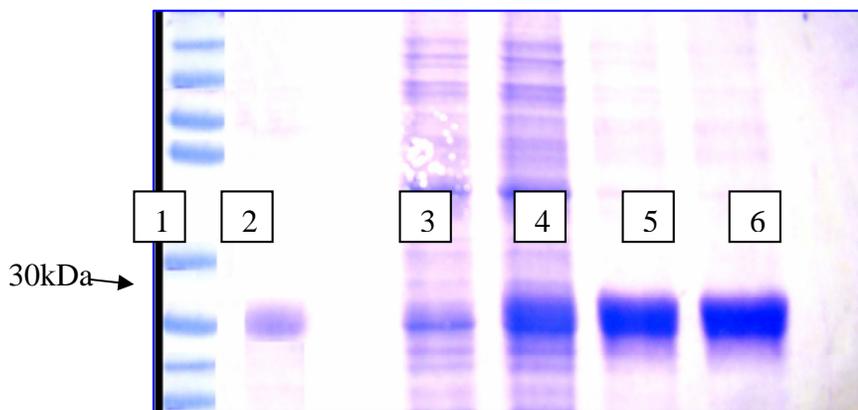


Figure.2 HRSV M protein bands in SDS PAGE



1: Represent protein ladder, 2: RSV M protein (control),
3: Extracted protein eluted in 150mM (IM), 4: Elution in 200mM (IM),
5: Elution in 300mM (IM), 6 : Elution in 500mM (IM).

The result is expression of a recombinant protein with a 6xHis or poly-His tag fused to its N- or C-terminus (Hengen, 1995). Expressed His-tagged proteins can be purified and detected easily because the string of histidine residues binds to several types of immobilized metal ions, including nickel, cobalt and copper, under specific buffer conditions. In either case, the tag provides a means of specifically purifying or detecting the recombinant protein without a protein-specific antibody or

probe (12). This basis for affinity purification is known as immobilized metal affinity chromatography (IMAC). resins such as Ni-NTA Superflow Agarose provide for purification of 1 to 80 milligrams of His-tagged protein per millilite Poly-His tags bind best to IMAC resins in near-neutral buffer conditions (physiologic pH and ionic strength). A typical binding/wash buffer consists of Tris-buffer saline (TBS) pH 7.2, containing 10-25mM imidazole. The low-

concentration of imidazole helps to prevent nonspecific binding of endogenous proteins that have histidine clusters of agarose beads. Nickel is the most widely available metal ion for purifying histidine-tagged proteins. Nickel generally provides good binding efficiency to His-tagged proteins but also tends to bind nonspecifically to endogenous proteins that contain histidine clusters. a small amount of imidazole in the binding/wash buffer helps to control off-target binding (Sugar and Neumann, 1984).

The RSV M protein gene cloned in PET16b was successfully transformed into *E.Coli* LB 21 . The transformed bacteria was able to express M protein in high concentration. The expressed protein was purified by using Ni-NTA Affinity Chromatography.

References

- Zambon, M.C., Stockton, J.D., Clewley, J.P. and Fleming, D.M. 2001. Contribution of influenza and respiratory syncytial virus to community cases of influenza-like illness: an observational study. *Lancet* 358, 1410–1416.
- Teresita, M.C., Allen, E.H., Tilli, L.H., Gurpreet, K.S., David, M., Ryan, L., Ann, M., Derric, H., Darry, A.K., Stephan, F., Tony, R.B., Richard, G.H., Delbert R.D. and George, R.A. 2013, Formation of stable mimic of ambient particulate matter containing infectious respiratory syncytial virus and its dry-deposition directly onto cell culture. *Anal.Chem.* 85, 898-906.
- Ghildyal, R., Mills, J., Murray, M., Vardaxis, N. and Meanger, J. 2002. Respiratory syncytial virus matrix protein associates with nucleocapsids in infected cells. *J Gen Virol* 83, 753–757.
- Teng, M.N. and Collins, P.L. 1998. Identification of the respiratory syncytial virus proteins required for the formation and passage of helper-dependent infectious particles. *J Virol* 72, 5707–5716.
- Oomens, G.P., Megaw, A.G. and Wertz, G.W. 2003. Infectivity of a human respiratory syncytial virus lacking the SH, G and F proteins is efficiently mediated by the vesicular stomatitis virus G protein. *J Virol* 77, 3785–3798.
- Henderson, G., Murray, J. and Yeo, R. 2002. Sorting of the respiratory syncytial virus matrix protein into detergent-resistant structures is dependent on the cell-surface expression of the glycoproteins. *Virology* 300, 244–254.
- Burke, E., Dupuy, L., Wall, C. and Barik, S. 1998. Role of cellular actin in the gene expression and morphogenesis of human respiratory syncytial virus. *Virology* 252, 137–148.
- Ulloa, L., Serra, R., Asenjo, A. And Villanueva, N. 1998. Interactions between cellular actin and human respiratory syncytial virus (HRSV). *Virus Res* 53, 13–25.
- Wakefield, L. and Brownlee, G. 1989. RNA-binding properties of influenza A virus matrix protein M1. *Nucleic Acids Res* 17, 8569–8580.
- Neumann, E., Schaefer-Ridder, M., Wang, Y and Hofschneider, P.H. 1982. Gene transfer into mouse lyoma cells by electroporation in high electric fields. *The EMBO Journal* 1 (7): 841–5.
- Hochuli, E., Bannwarth, W., Döbeli, H., Gentz, R. and Stüber, D. 1988. Genetic Approach to Facilitate Purification of Recombinant Proteins with a Novel Metal Chelate Adsorbent. *Nature Biotechnology* 6 (11): 1321–1325.
- Hengen, P. 1995. Purification of His-Tag fusion proteins from *Escherichia coli*. *Trends in Biochemical Sciences* 20 (7): 285–6.
- Sugar, I.P. and Neumann, E. 1984. Stochastic model for electric field-induced membrane pores electroporation. *Biophysical Chemistry* 19 (3): 211–25.