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Functional analysis of the L Protein of Nipah Virus Using Minigenome System

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ABSTRACT

Nipah virus (NiV) was identified in Malaysia in late September 1998 as the etiological agent of an outbreak of acute encephalitis with the high mortality rate in human. NiV polymerase gene (L) encodes RNA-dependent RNA polymerase (RdRp) which is an enzyme required for viral replication. The aim of this study is to investigate the role of the N terminal of polymerase protein in replication of NiV. Based on the extensive deduced amino acid sequence analyses of a number of L proteins of non-segmented negative-strand (NNS) RNA viruses, a cluster of high-homology sequence segments have been identified within the body of the L proteins. The functional characterization of the NiV L polymerase was addressed in this research by generating a series of progressive N- deletions in the cloned gene and testing them in a minigenome assay. Five mutations that delete increasing amounts of the amino terminus of the L protein (20 amino acids) were generated at the amino-terminal. The ability of new recombinants plasmids in viral replication were tested by using minigenome system which is based on an intracellular and plasmid-based replication assay. This system contains an inserted chloramphenicol acetyltransferase (CAT) gene, for readout of the replication of the mini genome directed by wild type or mutant L protein. The results demonstrated that the first 100 amino acids of the NH2-terminal domain spanning a highly conserved motif are directly involved in transcription of the genome RNA. The possible functional significance of the NH2-terminal domain of paramyxovirus L protein is discussed.

Keywords: L gene; polymerase protein; mutagenesis; Nipah virus

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INTRODUCTION

Nipah virus (NiV) is a recently emergent, highly pathogenic, zoonotic paramyxovirus in the genus Henipavirus within the family Paramyxoviridae (Mayo, 2002). Natural reservoir for NiV is a bat from the genus Pteropus which also known as fruit bat (K. B. Chua et al., 2002; S. A. Rahman et al., 2010; Reynes et al., 2005; Wacharapluesadee et al., 2005). Exposure to infected fruit bats; and other intermediate hosts such as infected pigs and/or in some cases direct contact with infected human are the various ways for human infection to NiV (Horikami, Smallwood, Bankamp, & Moyer, 1994; Lo et al., 2012; M. A. Rahman et al., 2012) As for other paramyxoviruses, NiV is an envelope, non-segmented, negative-sense RNA (NNS) virus. NiV genome is 18,246 nucleotides (nt) in length which is the largest genome within the family Paramyxoviridae (K. Chua et al., 2000; Harcourt et al., 2001). Viral genome contains six transcription units encode six structural proteins namely the nucleocapsid (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) and finally, the large protein (L) or the polymerase protein which are ordered in 3'-N-P-M-F-G-L -5' (Harcourt et al., 2005; Harcourt et al., 2000; Wang et al., 2001). The replication of genetic material of NNS viruses depends on a large multifunctional L polypeptide to catalyze many of the steps in RNA synthesis and processing. The viral RNA polymerase is constituted of two proteins, the large protein (L) and the phosphoprotein (P). The P protein plays an essential role in the enzyme; and in order to form an active polymerase complex, the L and P polypeptides must physically interact (Chattopadhyay & Shaila, 2004; Horikami et al., 1994). The Paramyxoviridae L protein is the least-characterized protein of the replicative complex and is also the biggest protein encoded by the viral

genome (2244 amino acids) (Halpin, Bankamp, Harcourt, Bellini, & Rota, 2004). Bioinformatics studies of all the L proteins of NNS viruses have revealed the presence of six highly conserved domains, I to VI, which are joined by variable non-conserved regions (Delarue, Poch, Tordo, Moras, & Argos, 1990; Harcourt et al., 2001). The functionally important regions of the polymerase proteins are viewed to reside within six linear domains where are conserved between all NNS viruses (Delarue et al., 1990; Harcourt et al., 2001). However, there are some areas of conservation which have been identified outside the six linear domains within the family Paramyxoviridae (Delarue et al., 1990). For instance, in NiV, there are 233 amino acids after the AUG codon to initiate the translation of the first domain in the L gene (Harcourt et al., 2001). From extensive deduced amino acid sequence analyses of the cDNA clones of a number of L proteins of NNS viruses, a cluster of high-homology sequence segments have been identified within the first 100 amino acids of the L proteins. The main purpose of this research is to analysis the role of this conserve domain regarding the replication of NiV. In general, the nature of viral polymerase protein is very important due to the lack of RNA-dependent RNA (RdRp) polymerases in human cells. This unique feature of RdRp polymerase proteins makes them a promising target for antiviral therapy. This study has focused on NiV polymerase protein; using serial deletion mutagenesis to identify the function of the conserved NH2-terminal domain of the NiV RNA polymerase. NiV minireplicon system was developed to measure the transcription of the NiV genome by the mutant L proteins. As it is shown in Figure 1, multiple alignment sequence (MSA) of the NiV L protein with eight other L proteins of paramyxoviruses was implemented using the CLUSTALW2 sequence analysis software. These paramyxoviruses are included: (Mumps virus[MU]; Simian virus 5 [SV5]; Human parainfluenza virus type 2 [PIV2]; Hendra virus[HeV]; Measles virus [MV]; Canine Distemper virus [CDV]; Sendai virus (SV); New Castle disease virus [NDV]); and lastly Nipah virus [NiV] L proteins. For the alignment, the minimum percent similarity score was 40% over a 100-amino-acid window, using an open gap penalty of 6.0. the asterisk"*" means that the residues or nucleotides in that column are identical in all sequences in the alignment. The colon":" implies that conserved substitutions have been observed. A full stop"." means that semi-conserved substitutions are observed. The amino-terminal sequences alignment demonstrated 7 conserved substitutions points and 4 semi-conserved substitutions in 9 paramyxovirus L proteins were aligned.

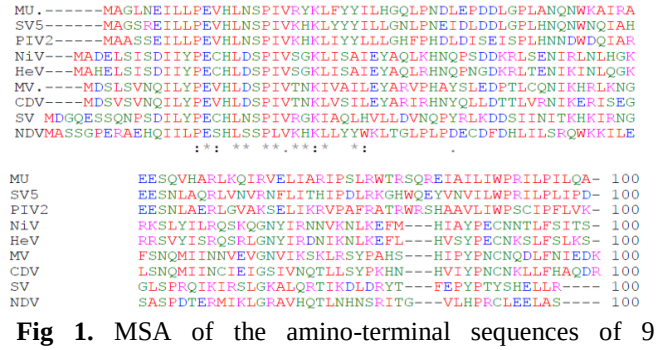

Fig 1. MSA of the amino-terminal sequences of 9 paramyxovirus L proteins.

The alignment revealed the presence of a highly conserved region in close proximity to the NH2 terminus between amino acid residues 13 to 25, with a unique sequence [IL(Y/L)PE(C/V)HL (N/D)SPIV] being highly conserved among the paramyxovirus L proteins. Similarly, it was shown by (Malur, Choudhary, De, & Banerjee, 2002) that there are only eight amino acids (residues 3 to 10) difference between Human parainfluenza virus type 3 (HPIV 3) and Sendai virus (SV) L protein within all of NH2-terminal residues 1 to 25. By generating serial deletions at the first 100 residues of the amino-terminus of NiV, this study is an effort to understand the role and importance of this domain. The ability of the mutant L proteins to transcribe a NiV minireplicon was examined. The results suggested that an NH2-terminal region of the NiV L protein (the first 100 amino acids) encompasses a highly conserved motif which is directly involved in the transcription of the genome and possibly in forming a functional complex with the phosphoprotein.

MATERIALS AND METHODS

Different types of plasmid were applied in this study which presented in Table 1. Briefly they are included: pMGNiVR6 or pNiV-CAT (the viral 3′ [leader] and 5′ [trailer] non-coding regions flanking either the chloramphenicol acetyltransferase) or (minigeneome), pNiV-N (nucleoprotein cloned into the pCITE), pNiV-P (phosphoprotein cloned into the pCITE), pNiV-L (polymerase protein cloned into the pCITE) and pTriEX-T7 were generously provided by Dr. Sheriff from the Universiti Kebangsaan Malaysia (UKM). The complete gene sequence of NiV was extracted from GenBank under accession number AF212302.2.

Table1. Different plasmids have been used in this study

Plasmid	Vector	Size (kb)	Inserted gene	Size (kb)
pMGNiVR6 or pNiV-CAT	pCITE-2α(+)	3.8	CAT gene	1.8
pNiV-N	pCITE-2α(+)	3.8	Nucleoprotein gene	2.3
pNiV-P	pCITE-2α(+)	3.8	Phosphoprotein gene	2.7
pNiV-L (wild-type)	pCITE-2α(+)	3.8	Polymerase gene	6.7
pNiV-L (mutated)	pCITE-2α(+)	3.8	Polymerase gene	various
pTriEX-T7	pTriEX-1.1	5.3	T7 gene	2.9

CONSTRUCTION OF PNIV-L MUTANTS

The areas of interest on the L gene were amplified using high fidelity PCR. The plasmid of the pNiV-L served as the template for introducing the mutations. Five serial deletions with 60 nt distance were produced at the amino terminus of the L gene. Also, the COOH terminus deletions were produced in the same way. These carboxyl deletions retained the NH2-terminal residues and used as a control for the experiment. Overall, ten deletion mutants, as it is shown in Figure 2, were constructed at the N and the C terminal of NiV large polymerase. Five sets of DNA oligonucleotide primers were designed at each ends of the L gene. Five serial forward

primers at the N terminal include:pN-60: 5'-GTC ATT TGG ACC ATG GTA TAG TCT CTG GTA-3'; pN-120: 5'-GAG ACA CAA CCA TGGGAG TGA TGA TAA AA-3'; pN-180: 5-'GGA AAA GCCATGGGC TAT ACA TAT TAA GAC-3'; pN-240: 5'- CAA CAT AAA CCA TGGGAA GGA ATT CAT GC-3'; pN-300: 5'-CTC CAT CCA TGGTCA AGG CAT GAC TAG C-3' which encoded NcoI restriction site (RE) were paired with reveres primer 5'- CAT CCG CGG CCG CTA AGT CTT TGT CAT G -3'which encoded NotI RE. Similarly, five forward primer pC-60: 5'-CTC GATGCG GCC GCTAAA CTA TCC AAA C-3'; pC-120: 5'- GCA TCCGCG GCCGCTAAG TCT TTG TC-3'; pC-180: 5'- CTT CGCGGC CGCTAATTG ATG TGA TAA AG- 3'; pC-240: 5'- TAA GTGGCG GCCGCT AAA CTT TTT TAG TCA C-3'; pC-300: 5'-TCC AGC GGC CGCTAAGGT TCT AGG TG- 3' which encoded NotI RE were designed at the C terminal and matched with reverse primer R-pN: 5'-CCA TGGGAT GGC CGA TGA ATT ATC AAT ATC C-3' with NotI RE. The Primers were 30-32 nucleotides long and the percentage of Guanine (G) and Cytosine (C) in the primer was assessed between 40-60%. The primers are designed to contain a complementary 5' sequence.

The length of the correctly matched sequence in the mutagenic primers (without restriction enzyme sequence) was approximately 23-27 nucleotides. The non-complementary region of primers consisted of a restriction enzyme and an additional base in one of the primers to keep the polymerase reading frame between the restriction enzyme cutting sequence and the rest of the primer. pfu DNA polymerase (Promega) was used to amplified the desirable sequence. PCR products were purified from standard agarose gels in TBE buffer (50 mM Tris, 50 mM boric acid, 0.2 mM EDTA [acid form, ~pH 8.5]) by QIAquick gel extraction kit (QIAGEN) using manufacturer's instruction.

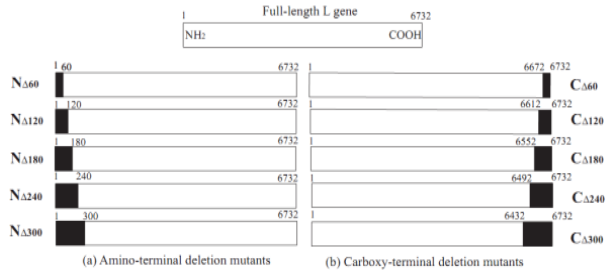

Figure2. Schematic representations of the NiV-L constructed deletion mutants. The names of deletion mutants were according to the location of mutation (amino- or carboxyl terminus) and amino acids removed by the mutagenesis procedure. Solid boxes indicate deleted regions and the amino acid positions at the deleted regions were shown by digits.

The DNA fragments were inserted into the NcoI-NotI site of plasmid pCITE-2α (Novagen) (originally published as pE5LVPO was made by Griff Parks, Greg Duke and Ann Palmenberg at the University of Wisconsin) to obtain expression vector pCITE. Verification of the targeted sequence and the orientation of the gene DNA were executed by sequencing procedure.

IN VITRO TRANSCRIPTION/TRANSLATION

The In Vitro synthesis of proteins in this study was performed by TNT® Quick Coupled Transcription/Translation Kit (Promega) which is a cell-free translation system consists of extracts from rabbit reticulocytes. 4µl of new recombinant plasmids (0.5 µg/µl) was added to 40 µl of TNT® Quick Master Mix in the presence of 1 µl methionine and 4 µl Transcend-bioyn-lysyle (tRNA). Total volume adjusted to 50µl by adding nuclease-free water and for 90 min at 30 °C samples were incubated. The proteins were labeled with biotinylated lysine and the biotinylated proteins were detected by methods similar to those used in Western Blotting.

Proteins were seperated by 5% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose filters. The filters were soaked for overnight with 10% skimed milk then they were washed three times by TBS-T (0.05 M Tris buffer (pH 8.6), 0.5 M NaCI and 0.5% Triton X-100). The filters were incubated in diluted Streptavidin-HRP in TBS-T (1:5000) for one hour while they shook gently. After another washing step by TBS-T; chemiluminescent substrates for HRP were added. These substrates comprise of a stable peroxide solution or substrate A and an enhanced luminol solution or substrate B. Blots were developed with 2.5 ml of each substrates solution diluted in 15 ml double distilled water (ddH2O). After 2 min incubation; the membranes were placed on transparency film and put it inside a film cassette and expose the blot to Kodak X-omat AR for 30 min and develop the film.

TRANSFECTION OF NEW RECOMBINANT PLASMIDS

BHK-21 cells (approximately 1×10^4cells/mL) were plated into 18mL of Dulbecco's Modified Eagle medium (Gibco) supplemented with 10% (v/v) fetal bovine serum (PAA) and grown to 70-75% confluence in T-75 culture flask by incubating the cells at 37º C in 5% carbon dioxide condition. Every new recombinant plasmids including: pNiV-NΔ60 pNiV-NΔ120 pNiV-NΔ180 pNiV-NΔ240 pNiV-NΔ300, pNiV-CΔ60 pNiV-CΔ120 pNiV-CΔ180 pNiV-CΔ240 pNiV-CΔ300; were co-transfected with supporting plasmids (pNiV-N, pNiV-P), pTriEX-T7 and minigenome (pNiV-CAT) into the cell culture. The greatest amounts of produced CAT were determined by conducting a series of titration experiments.

For the standard assay, the cells in each flask were transfected with 1.5 µg pNiV-N, 0.5 µg pNiV-P, 1.0 µg of new constructed L mutants, 1.0 µg pTriEX-T7 and 1.0 µg minigenome plasmid (pNiV-CAT) by using the GeneJuice Transfection Reagent (Merck). Based on the manufacturer's directions, 2 µl of transfection reagent was used for each microgram of transfected DNA. For experimental controls, the NiV-L wild-type (pNiV-Lwt) was applied as positive control. Furthermore, the N gene of NiV (pNiV-N) was removed from supporting plasmids; providing negative control (pNiV-ΔN).

The cells were subsequently incubated at 37º C in the presence of 5% carbon dioxide for a total of 48h. The cells were then harvested to measure their CAT activity. Each transfection reaction was set up in duplicate.

CAT ELISA

The cells extractions were prepared 48 h after transfection in 1 ml of lysis buffer (50 mM tris (pH 7.4), 1% Triton X-100,1 mM EDTA, 1 mM EGTA, 50 mM NaF, 0.2 mM sodiumorthovanadate, 0.2 mM phenylmeth- ylsulfonyl fluoride and 0.5% NP-40). The concentrations of CAT protein in the extracts were measured with the CAT ELISA kit (Roche) and the amounts of extracts used per sample were adjusted for protein concentration to ensure comparable results. Optical densities were determined in the Rainbow ELISA reader at 405 nm. The efficiency of viral transcription activity of the mutated L genes was measured by the CAT protein levels relative to wild-type of polymerase protein.

WESTERN BLOT ANALYSIS

The CAT protein in the harvested cells was visualized by western blot. The total protein of cell extract was separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose filters. The filters were soaked for overnight with 10% skimed milk and then they were washed three times by TBS-T (0.05 M Tris buffer (pH 8.6), 0.5 M NaCI and 0.5% Triton X-100). CAT protein was detected by primary antibody anti-CAT-DIG followed by secondary antibody anti-DIG-POD, which were provided by CAT ELISA kit (Roche). The membranes were placed on transparency film and put them inside a film cassette and exposed the blot to Kodak X-omat AR for 30 min. The films were developed.

RESULTS

The RNA dependent RNA polymerase (RdRp) consists of a complex of the large (L) and phosphoprotein (P) subunits where L is thought to be responsible for all the catalytic activities necessary for viral RNA synthesis. The P-L interaction has been extensively studied for other viruses such as Sendai virus and Rinderpest viruses (Chattopadhyay & Shaila, 2004). Also the binding sites in both proteins have been identified. The L binding site was mapped to be at the N terminal of L protein (Çevik, Smallwood, & Moyer, 2003). The purpose of this study was to probe into the structure and function of the L protein in order to corroborate the presence of such domain in newly emerge virus NiV. The NΔ60 NΔ120 NΔ180 NΔ240 and NΔ300 truncations deleted 60, 120, 180, 240 and 300 amino acids, respectively, from the N terminal end of the 2244 amino acid L protein. Deletions were designed to be multiple of six because NiV such as other members of Paramyxovirinae replicate efficiently only when their genomes are integer hexamer length (Calain & Roux, 1993; Halpin et al., 2004; Kolakofsky et al., 1998)

GENERATION OF PNIV-L MUTANTS

A total of ten deletion mutants located at the amino- and the carboxy-terminal of pNiV-L were constructed as described (Figure 1). Two sets of serial deletion mutants were constructed in which 20,40,60,80 and 100 amino acids were deleted from either the N- or the C-terminus. Truncated L genes were successfully cloned into cloning vector pCITE-2a and eventually 10 new recombinant plasmids were constructed (Figure 3). The size of vector (pCITE) is 3.8 kb and length of the L gene of NiV is (6.7 kb). Anticipated length of new recombinant plasmid is ~10.5 kb.

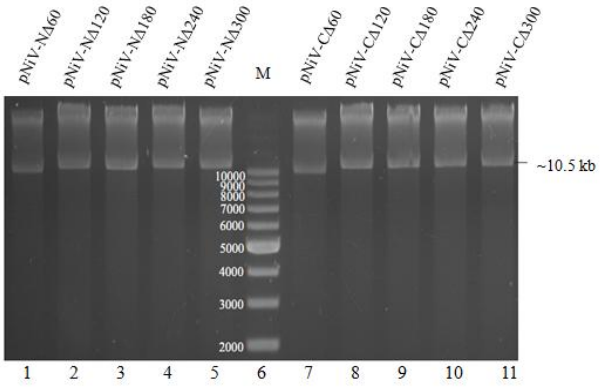

Figure 3. Agarose gel electrophoresis (1%) of the constructed plasmids. Lane 1-5: represent the new recombinant plasmids at the N terminal and lane 7-11 show the c terminal mutant plasmids. M represents Promega Supercoiled DNA Ladder.

IN VITRO TRANSCRIPTION/TRANSLATION (IVTT)

In vitro functional analysis of ten new recombinant plasmids was monitored and the results reflect the facts that all truncated genes are able to be expressed in vitro. In order to detect specificity of proteins gained in vitro a chemiluminescence western blotting was implemented. As the results demonstrated in Figure 4; all new recombinant polymerase plasmids from Nipah virus are able to in vitro synthesis of proteins in cell-free translation system. The results reflect the fact that new recombinant templates are able to express despite the deletion introduced into NiV L gene.

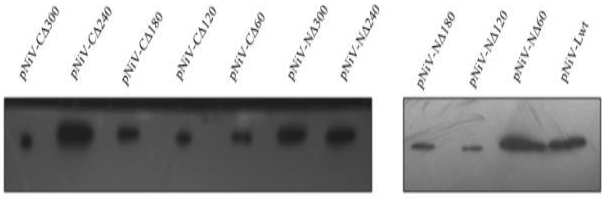

Figure 4. Chemiluminescent Detection of IVTT products.

FUNCTIONAL ANALYSIS OF MUTATED L PROTEINS

The NiV minireplicon system was previously shown to serve as an efficient assay to measure the transcription efficiency of NiV genome. In this test quantitative CAT reporter gene expression was measured in the presence of exogenous support plasmids encoding N, P and L. The ability of the mutated L proteins in association with P and N protein to transcribe a NiV minigenome (pNiV-CAT) was evaluated. For this assay, BHK-21 cells were infected with a plasmid cocktail includes minigenome, the support plasmids and plasmid encoding T7 RNA polymerase resulted in RNA replication and transcription of the minigenome. The co-transfected support plasmids carrying N, P and L (wild-type [wt] or mutant) gene of NiV individually were transcribed

under the control of the T7 promoter. They generate the RNP template which is replicated and transcribed by the intracellularly reconstituted virus polymerase. The synthesized full-length of antigenomic RNA will then be encapsidated and served as template for the synthesis of progeny genomic RNA. Hence, CAT gene mRNA strands will be synthesized and subsequently were translated into the CAT protein.

MEASUREMENT OF CAT EXPRESSION

Detection of CAT protein reflects the synthesis of CAT mRNA from the minigenome, showing that the minigenome system is functioning and be activated in the host cells. In this study CAT ELISA Kit from Roche Applied Science was used to detect the levels of CAT expression. The kit follows a standard ELISA protocol in microplates. Antibodies to CAT (anti-CAT) are prebound to the surface of the microplates. Lysis of the transfected cells, the cell extracts, which contained CAT enzyme, was added to the wells of the microplate modules. Specifically binding of all CAT contained in the cell extracts to the anti-CAT antibodies bound to the microplate surface. The absorbance of the samples was determined using a microplate (ELISA) reader which directly correlated to the level of CAT present in the medium supernatant. The absorbance measured for each sample at 405 nm (reference wave length: approx.490 nm). Calculation of CAT ELISA results are based on the principle of a solid phase enzyme-linked immunosorbent assay. Initially, average absorbance values for each set of duplicate standards and duplicate samples were calculated. Results of average absorbance value are presented in Table 2.

Table 2. Absorbance value for each sample. Negative control in this study was provided by removing pNiV-N plasmid from the supporting plasmids in minigeneome (pNiV-ΔN) and wild type of L gene (pNiV-Lwt) was applied as positive control.

New Recombinant Plasmid	N-Terminal	New Recombinant Plasmid	C-Terminal
pNiV-NΔ60	0.516	pNiV-CΔ60	2.489
pNiV-NΔ120	0.520	pNiV-CΔ120	2.011
pNiV-NΔ180	0.516	pNiV-CΔ180	2.298
pNiV-NΔ240	0.547	pNiV-CΔ240	1.978
pNiV-NΔ300	0.532	pNiV-CΔ300	2.315

Absorbencies were recorded for CAT Standard concentrations of 0.125, 025, 0.5, 1.0 and 0.0ng/pl in a sample volume of 200µl. Subsequently, a standard curve by plotting the mean absorbance for each standard concentration (x axis) against the target protein concentration (Y axis) was created. The standard curve was generated to evaluate the concentration of CAT enzyme in each sample. Basically, the minireplicon is subsequently transcribed by support plasmid-encoded proteins to generate the CAT mRNA that are translated into the CAT protein.

Cytoplasmic extracts prepared from such infected BHK-21 cells were used to determine the CAT activity as described in Materials and Methods. 48 h after transfection of mutated L gene along with the support plasmids the level of CAT concentration in cytoplasmic extracts for each mutant was assessed by ELISA. The levels of expression of the CAT reporter gene by the mutants; wild type; and negative control are shown in Figure 5.

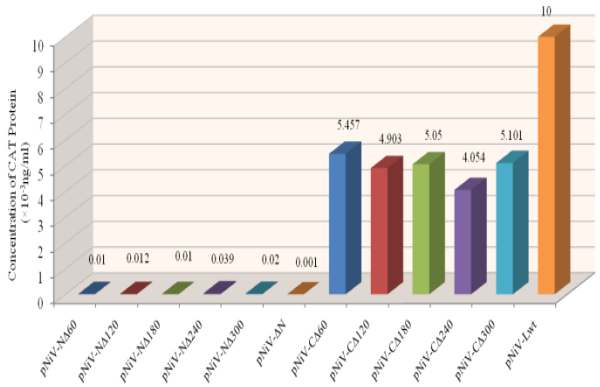

Figure 5. Levels of expression of the CAT reporter gene from different mutated L plasmids. pNiV-ΔN represented the sample in which the pNiV-N was omitted in transfection mixtures and it applied as negative control. Also, pNiV-L wild-type was mixed in the plasmids cocktail and employed as positive control.

As determined by the lack of detectable CAT activity, the mutations introduced in the amino-terminus of the L protein were virtually inactive while the carboxyl mutants (ΔC) retained more than 50% CAT activity compared to that of the wild type.

WESTERN BLOT

To provide supporting data and strengthening our results we executed western blot. The transfection products were blotted from the SDS-polyacrylamide gel on nitrocellulose membrane by using standard apparatus and protocol. The results of SDS-PAGE analysis are presented in Figure 6.

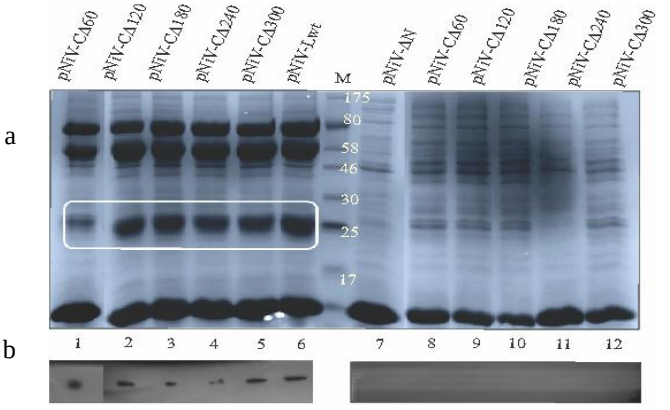

Figure 6. Western blotting analysis of CAT expression of mutant samples. Marker is Prestained Protein Marker (New ENGLAND BioLabs® Inc.)

The total protein of the cell lysates samples were separated by SDS polyacrylamide gel electrophoresis. CAT protein molecular weight is 25 kDa. CAT protein in the SDS-PAGE pattern of 5 serial mutants at the C terminal (lane 1-5)and positive control pNiV-L wild-type (lane 6) was detected and absence of CAT protein in examined samples at the N terminal (lane 8-12) and negative control pNiV-ΔN (lane 7) was verified. The part (b) of the Figure 6, displays result of western blotting performed on the C and the N terminal. There are no bands present at the amino-terminus of the L gene and negative control while there are distinctive bands at the carboxyl-terminus and positive control (lane 1-6). It is noticeable that progressive deletion at the C terminal from the full-length L protein (C-2244-2144) caused only reduction in polymerase activity (~50%). These deleted polymerase genes which retained their amino-terminus residues; used as control and showed that the carboxyl-terminus 100 non-conserved residues are dispensable for the transcriptive function of the L protein.

DISCUSSION

The main focused in this report was on yet another conserved domain presents at the NH2 terminus of the NiV L protein. Methodology which applied in this research was based on mutational analysis. Progressive deletions within this conserved domain were created and the function of these deleted sequences in replication of virus was analyzed. The results demonstrated that an NH2-terminal region of the NiV L protein comprising 100 amino acids which encompass a highly conserved motif is directly involved in the transcription of genome RNA and possibly in forming a functional complex with the phosphoprotein (P). However, it is possible that the NH2-terminal 100 amino acids are critical in providing optimal conformation to the L protein, including a highly ordered structure required for efficient transcription and P binding. Based on this theory, the NH2-terminal region of the L protein may be the exact location of phosphoprotein binding. Hence, any deletion within the N-terminal 100 amino acids (>5% of the full L gene) stretch will result in a misfolding of the polymerase's structure and eventually cause the loss of P binding ability. Overall, the defect in the ability of these N-terminal deletion mutants to interact with P could reflect three different possibilities including: first, the P-binding site has been masked rather than deleted; second these alterations have induced a global malfolding of L and finally a more interesting possibility is that the N-terminal site for P binding is composed of widely dispersed discontinuous regions in L that fold into a secondary structure easily disrupted by deletions. Although extensive mutational analyses have not been implemented within the rest of the highly conserved sequence of L, it is tempting to speculate that the 100 NH2-terminal amino acids of other paramyxoviruses may also play a critical role in the activity of the corresponding L protein. In summary, current study has shown a highly conserved domain within the NH2 terminus of the NiV L protein that is directly involved in viral transcription possibly plays a critical role in complex formation with the P protein. Further research is also required to develop a deeper understanding of the possible functional significance of the NH2-terminal domain of paramyxovirus L protein, and ultimately applying it as targets for antiviral therapy.

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REFERENCES

Calain, P., & Roux, L. (1993). The rule of six, a basic feature for efficient replication of Sendai virus defective interfering RNA. Journal of virology, 67(8), 4822-4830.

Çevik, B., Smallwood, S., & Moyer, S. A. (2003). The L–L oligomerization domain resides at the very N-terminus of the sendai virus L RNA polymerase protein. Virology, 313(2), 525-536.

Chattopadhyay, A., & Shaila, M. (2004). Rinderpest virus RNA polymerase subunits: mapping of mutual interacting domains on the large protein L and phosphoprotein P. Virus genes, 28(2), 169-178.

Chua, K., Bellini, W., Rota, P., Harcourt, B., Tamin, A., Lam, S., Shieh, W.-J. (2000). Nipah virus: a recently emergent deadly paramyxovirus. Science, 288(5470), 1432-1435.

Chua, K. B., Lek Koh, C., Hooi, P. S., Wee, K. F., Khong, J. H., Chua, B. H., Lam, S. K. (2002). Isolation of Nipah virus from Malaysian Island flying-foxes. Microbes and Infection, 4(2), 145-151.

Delarue, M., Poch, O., Tordo, N., Moras, D., & Argos, P. (1990). An attempt to unify the structure of polymerases. Protein Engineering, 3(6), 461-467.

Halpin, K., Bankamp, B., Harcourt, B. H., Bellini, W. J., & Rota, P. A. (2004). Nipah virus conforms to the rule of six in a minigenome replication assay. Journal of general virology, 85(3), 701-707.

Harcourt, B. H., Lowe, L., Tamin, A., Liu, X., Bankamp, B., Bowden, N.,Hossain, M. J. (2005). Genetic characterization of Nipah virus, Bangladesh, 2004. Emerging infectious diseases, 11(10), 1594.

Harcourt, B. H., Tamin, A., Halpin, K., Ksiazek, T. G., Rollin, P. E., Bellini, W. J., & Rota, P. A. (2001). Molecular characterization of the polymerase gene and genomic termini of Nipah virus. Virology, 287(1), 192-201.

Harcourt, B. H., Tamin, A., Ksiazek, T. G., Rollin, P. E., Anderson, L. J., Bellini, W. J., & Rota, P. A. (2000). Molecular characterization of Nipah virus, a newly emergent paramyxovirus. Virology, 271(2), 334-349.

Horikami, S. M., Smallwood, S., Bankamp, B., & Moyer, S. A. (1994). An amino-proximal domain of the L protein binds to the P protein in the measles virus RNA polymerase complex. Virology, 205(2), 540-545.

Kolakofsky, D., Pelet, T., Garcin, D., Hausmann, S., Curran, J., & Roux, L. (1998). Paramyxovirus RNA synthesis and the requirement for hexamer genome length: the rule of six revisited. Journal of virology, 72(2), 891-899.

Lo, M. K., Lowe, L., Hummel, K. B., Sazzad, H. M., Gurley, E. S., Hossain, M. J.,. Rollin, P. E. (2012). Characterization of Nipah virus from outbreaks in Bangladesh, 2008–2010. Emerging infectious diseases, 18(2), 248.

Malur, A. G., Choudhary, S. K., De, B. P., & Banerjee, A. K. (2002). Role of a highly conserved NH2-terminal domain of the human parainfluenza virus type 3 RNA polymerase. Journal of virology, 76(16), 8101-8109.

Mayo, M. (2002). A summary of taxonomic changes recently approved by ICTV. Archives of virology, 147(8), 1655-1656.

Rahman, M. A., Hossain, M. J., Sultana, S., Homaira, N., Khan, S. U., Rahman, M., Comer, J. A. (2012). Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. Vector-Borne and Zoonotic Diseases, 12(1), 65-72.

Rahman, S. A., Hassan, S. S., Olival, K. J., Mohamed, M., Chang, L.-Y., Hassan, L.,Naim, M. (2010). Characterization of Nipah virus from naturally infected Pteropus vampyrus bats, Malaysia. Emerging infectious diseases, 16(12), 1990.

Reynes, J.-M., Counor, D., Ong, S., Faure, C., Seng, V., Molia, S., Sarthou, J.-L. (2005). Nipah virus in Lyle's flying foxes, Cambodia. Emerging infectious diseases, 11(7), 1042.

Wacharapluesadee, S., Lumlertdacha, B., Boongird, K., Wanghongsa, S., Chanhome, L., Rollin, P., Hemachudha, T. (2005). Bat Nipah virus, Thailand. Emerging infectious diseases, 11(12), 1949.

Wang, L.-F., Harcourt, B. H., Yu, M., Tamin, A., Rota, P. A., Bellini, W. J., & Eaton, B. T. (2001). Molecular biology of Hendra and Nipah viruses. Microbes and Infection, 3(4), 279-287.

