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In-Silico Analysis of Polymerase Basic Protein 2 in Different Strains of Influenza A Virus

Tariq Ahmad Masoodi[1*], Gowhar Shafi[2] and Anjana Munshi[3]

[1]Colllge of Applied Medical Sciences,
[2]Molecular Cancer Biology Research Lab.
[1,2]Department of Food Science and Nutrition, King Saud University, Riyadh Saudi Arabia
[3]Department of Molecular Biology, Institute of Genetics and Hospital for Genetic Diseases, Hyderabad, India.
**Corresponding Author E-mail: tahamasoodi@gmail.com*

Abstract

Influenza virus causes febrile respiratory illness. The infection results in significant mortality, morbidity and economic disruption. Here in this work, PB2 (polymerase basic 2) protein of H1N1 influenza A virus strain was studied for mutational analysis. It was found that this strain has undergone several changes at different base pairs in comparison to its homologous strains. Prediction of secondary structures (SOPMA) and PROSITE motif assignments were performed to gain functional insights. This in silico study revealed new motifs at positions 471-473, 661-663, 684-689 and 717-720. These motifs were formed due to the mutations at position 473 (M473K), 661 (V661S), 684 (A684G) and 717 (A717T) respectively. The mutation A684G was found to be important in the N-myristoylation motif of A/swine/Indiana/1726/1988 (H1N1) isolate. Moreover, effect of mutations on the secondary structures among strains is discussed.

Keywords: PB2 protein, H1N1, Mutation, Insilico analysis.

Introduction

Human viral influenza [1-3] is an acute respiratory disease which is rooted in the distant past and is one of the most important infectious diseases of mankind. The disease is highly contagious, with person to person spread by aerosol droplets which mainly infect the epithelial cells of the respiratory tract. The means to understand influenza and its epidemiology was provided in the late 1920s by the breakthrough

discovery of transmissibility of swine influenza in pigs by a filterable agent [4], and was followed by the discoveries of human influenza type A in 1933 [5] of B in 1940 [6], and of C in 1950 [7]. All human influenza viruses [1,8,9] belong to the family Orthomyxoviridae. There are three genera, corresponding to influenza types A, B, and C. Influenza A viruses, uniquely, are divided into subtypes based on major antigenic specificities of their hemagglutinin (HA) and neuraminidase (NA) proteins.

Type A is the most important of the influenza viruses, causing alternate annual outbreaks and epidemics during the winter seasons of the northern and the southern hemispheres [10]. Virus types B and C are of lesser importance than A. Pandemics of influenza A have occurred about three times per century since 1700 [11] and were manifested by worldwide spread of the disease, typically with high morbidity and mortality. The most extreme pandemic in the 20th century was that of Spanish influenza of 1918–1919, which killed an estimated 20–40 million persons throughout the world [11-14].

The importance of influenza viruses as worldwide pathogens in humans, domestic animals, and poultry is well known. Influenza A viruses have a segmented genome of single stranded negative-sense RNA. [15]. New strains of influenza viruses emerge each year that have been isolated from a variety of animals, including humans, pigs, horses, sea mammals, and birds. [16] Infection of poultry with highly pathogenic avian influenza virus can be devastating in terms of flock morbidity and mortality, economic loss and social disruption. [17] Influenza pandemics occur from genetic reassortment between avian and human influenza viruses or alternatively by the direct adaptation of avian influenza viruses to human influenza virus which may cause human-to-human transmission. [16]

The recent epidemic of the "2009 H1N1" influenza A virus (also called swine or Mexican flu) has put the world on alert since a new swine flu strain (naturally hosted by pigs) has crossed the species barrier to human and, apparently acquired the capability for human to human transmission [18,19]

Influenza A virus contains 8 segments of linear, negative-sense, single stranded RNA. In virus particles, the genomic RNAs (vRNAs) are associated with the RNA-dependent RNA polymerase proteins and the nucleoprotein (NP), which together forms the ribonucleoprotein (RNP) complexes [20]. The RNA polymerase consists of PB2, PB1, and PA proteins, encoded by the viral genome segments 1, 2, and 3, respectively [21]. After virus adsorption to and penetration into the host cell, the RNP complexes migrate into the nucleus, where transcription and replication of the viral genome take place. In the nucleus of the infected cell, three types of viral RNA are synthesized: the mRNA, synthesized from each vRNA, which contains a cap structure at the 50 terminus and poly A tail at the 30 terminus (transcription); the positive-sense cRNA, lacking a cap structure and polyA tail, synthesized from the vRNA; and vRNA synthesized using the cRNA as a template (replication) [22-25]. Thus, the RNA polymerase complex is integral to both transcription and replication of the vRNAs.

The polymerase protein PB2 is 759 residues long in influenza-A viruses. The polymerase complex is formed from protein subunit interactions with the PB1 subunit [26]. PB2 interacts with NP and not with PA [27, 28]. This subunit plays an important role in transcription of mRNA by binding with the 5’ methylated cap of pre-mRNA in

host cells for providing primers to viral mRNA synthesis [29]. PB2 is a well-documented component of the viral polymerase complex required for virus replication. The PB2 protein has been shown to be involved in host range restriction and pathogenicity [30, 31].

Materials and Methods

Data set

Polymerase basic protein of Influenza A Virus sub-type A/swine/Indiana/1726/1988 (H1N1) from chicken was analyzed in the present study. This protein sequence is available at NCBI with accession number [GenBank: ACQ84506]. The PB2 protein sequences of subtypes H5N1 and H1N1 were downloaded from Genome directory of INFLUENZA at NCBI [ftp://ftp.ncbi.nih.gov/genomes/INFLUENZA] and summary of sequence data is given in Table 1. The dataset consists of PB2 sequences from 3 H1N1 strains and 2 H5N1 strains. CLUSTALW was used [http://www.ebi.ac.uk/Tools/clustalw] for generating a multiple sequence alignment, MSA of 5 PB2 sequences.

Table 1: Strains selected for comparative study.

GenBank accession	Strain	Subtype	Source
ACQ84506	A/Indiana/1726/88	H1N1	Swine
ABS49953	A/Italy/671/1987	H1N1	Swine
ABB20386	A/AUS/749/80	H1N1	Duck
ABF56657	A/PEV16T/05	H5N1	Human
ABJ16817	A/Afghanistan/1207/06	H5N1	Chicken

Mutational analyses

The MSA was used to identify mutations in PB2 with reference to A/swine/Indiana/1726/1988 (H1N1). The amino acids sequences 401 to 750 were analysed in this study.

Secondary structure prediction

SOPMA tool at EXPASY was used to assign secondary structures to PB2 sequences [http://npsa-pbil.ibcp.fr].

Motifs search

PROSITE scan at the EXPASY was used to identify PROSITE motifs in PB2 protein [http://www.expasy.ch/tools/scanprosite/].

Results

Sequence analyses and Secondary Structure Prediction

The PB2 protein sequence of A/swine/Indiana/1726/1988(H1N1) isolate was compared with other homologous sequences as shown in Figure 1. The sequences have at least 95% similarity among themselves and a multiple sequence alignment is obtained using the software ClustalW (Figure 1). Mutations are summarized in Table 2 with changes in corresponding predicted secondary structures.

On comparing sequence of H1N1 protein of A/swine/Indiana/1726/1988 (H1N1) with the other homologous sequences, it was observed that most of the mutations are synonymous and does not change the secondary structure of the protein. Only one non synonymous mutation, A684G in A/swine/Indiana/1726/1988 was found to change the secondary structure from Helix to Coil at this site. Mutations at positions M473K, V661S and A717T are also identical in all the strains used in this study (Table 2). All these mutations changed the chemical property from hydrophobic to hydrophilic but the secondary structure remains the same. The most important finding observed was the identification of new motifs at 473 (TeK) and 661 (StK) in *Protein kinase C phosphorylation* motif, at 684 (GGveSA) in *N-myristoylation* motif, and 717 (TkgE) in *Casein kinase II phosphorylation* motif of A/swine/Indiana/1726/1988 (H1N1) as shown in Table 3.

```
.........I.............................V..... 450  A/Afghanistan/1207/06
.........I.................T.............V..... 450  A/PEV16T/05
.........I.............................V..... 450  A/AUS/749/80
.........I.............................V.T.... 450  A/Italy/671/1987
AMVFSQEDCMVKAVRGDLNFVNRANQRLNPMHQLLRHFQKDAKILFQNWG 450  A/Indiana/1726/88

T.P.............M.....T.L..V.V..................... 500  A/Afghanistan/1207/06
..P.............M.....M.L..V.V..................... 500  A/PEV16T/05
..P.............M.....M.L..V.V.............I...... 500  A/AUS/749/80
..P.............M.....M.L..V.V..A.................. 500  A/Italy/671/1987
IESIDNVMGMIGILPDLTPSTEKSMRGIRISKMGVDEYSSTERVVVSIDR 500  A/Indiana/1726/88

.......R.......................................... 550  A/Afghanistan/1207/06
.......R.......................................... 550  A/PEV16T/05
.......R.......................................... 550  A/AUS/749/80
.......R.......................................... 550  A/Italy/671/1987
FLRVRDQQGNVLLSPEEVSETQGTEKLTITYSSSMMWEINGPESVLVNTY 550  A/Indiana/1726/88

................................................. 600  A/Afghanistan/1207/06
................................................. 600  A/PEV16T/05
........I........................................ 600  A/AUS/749/80
....K............................................ 600  A/Italy/671/1987
QWIIRNWETVKIQWSQDPTMLYNKMEFEPFQSLVPKAARGQYSGFVRTLF 600  A/Indiana/1726/88

................................................. 650  A/Afghanistan/1207/06
.............................................V. 650  A/PEV16T/05
.............V...........E.....................V. 650  A/AUS/749/80
........................V........................ 650  A/Italy/671/1987
QQMRDVLGTFDTVQIIKLLPFAAAPPKQSRMQFSSLTVNVRGSGMRILIR 650  A/Indiana/1726/88

.........A.............T.......A.............K. 700  A/Afghanistan/1207/06
.........A.............T.......A.............K. 700  A/PEV16T/05
.........A.............T.......A.............K. 700  A/AUS/749/80
.........V.............T.......A.............K. 700  A/Italy/671/1987
GNSPVFNYNKSTKRLTVLGKDAGALNEDPDEGTGGVESAVLRGFLILGRE 700  A/Indiana/1726/88

.............N.A................................ 750  A/Afghanistan/1207/06
.............N.A................................ 750  A/PEV16T/05
.............N.A................................ 750  A/AUS/749/80
N............N.A................................ 750  A/Italy/671/1987
DKRYGPALSINELSSLTKGEKANVLIGQGDVVLVMKRKRDSSILTDSQTA 750  A/Indiana/1726/88
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Figure 1: Multiple sequence alignment of PB2 protein sequences (residue positions 401 to 750) of H1N1 and H5N1. Identical residues to A/Indiana/1726/88 are indicated by dots.

Table 2: Amino acid mutations in PB2 for different strains in comparison to A/Indiana/1726/88(H1N1). (H=helix, E=extended strand, C=random coil, T=turn).

Base change	Change in A/Indiana/1726/88	Change in properties	change in Secondary structure
A/Italy/671/1987			
411	I→V	Hydrophobic	H
444	V→I	Hydrophobic	H
446	T→F	Hydrophilic→Hydrophobic	H
453	P→S	Hydrophilic	C
467	M→L	Hydrophobic	C
473	M→K	Hydrophobic→Hydrophilic	H
475	L→M	Hydrophobic	H
478	V→I	Hydrophobic	E
480	V→I	Hydrophobic	E
483	A→M	Hydrophobic	T
508	R→Q	Hydrophilic	T
555	K→R	Hydrophilic	H
627	V→K	Hydrophobic→Hydrophilic	C→T
661	V→S	Hydrophobic Hydrophilic	T
676	T→N	Hydrophilic	C
684	A→G	Hydrophobic→Hydrophilic	H→C
699	K→R	Hydrophilic	C
701	N→D	Hydrophilic	C
715	N→S	Hydrophilic	
717	A→T	Hydrophobic→Hydrophilic	
A/AUS/749/80			
411	I→V	Hydrophobic	H
444	V→I	Hydrophobic	H
453	P→S	Hydrophilic	C
467	M→L	Hydrophobic	C
473	M→K	Hydrophobic→Hydrophilic	H
475	L→M	Hydrophobic	H
478	V→I	Hydrophobic	E
480	V→I	Hydrophobic	E
508	R→Q	Hydrophilic	T
559	I→T	Hydrophobic→Hydrophilic	C
615	V→I	Hydrophobic	H
627	E→K	Hydrophilic	T
649	V→I	Hydrophobic	E
661	A→S	Hydrophobic→Hydrophilic	T
676	T→N	Hydrophilic	C
684	A→G	Hydrophobic→Hydrophilic	H→C
699	K→R	Hydrophilic	C
715	N→S	Hydrophilic	H
717	A→T	Hydrophobic→Hydrophilic	C
A/PEV16T/05			
411	I→V	Hydrophobic	H
430	T→P	Hydrophilic	H
453	P→S	hydrophilic	C
467	M→L	Hydrophobic	C
475	L→M	Hydrophobic	H
478	V→I	Hydrophobic	E
480	V→I	Hydrophobic	E
508	R→Q	Hydrophilic	T
661	A→S	Hydrophobic→Hydrophilic	T
676	T→N	Hydrophilic	C
684	A→G	Hydrophobic→Hydrophilic	H→C
699	K→R	Hydrophilic	C
715	N→S	Hydrophilic	H
717	A→T	Hydrophobic→Hydrophilic	C
A/Afghanistan/1207/06			
411	I→V	Hydrophobic	H
444	V→I	Hydrophobic	H
451	T→I	Hydrophilic→Hydrophobic	C
453	P→S	Hydrophilic	C
467	M→L	Hydrophobic	C
473	T→K	Hydrophilic	H
475	L→M	Hydrophobic	H
478	V→I	Hydrophobic	E
480	V→I	Hydrophobic	E
508	R→Q	Hydrophilic	T
661	A→S	Hydrophobic→Hydrophilic	T
676	T→N	Hydrophobic→Hydrophilic	C
684	A→G	Hydrophobic→Hydrophilic	H→C
699	K→R	Hydrophilic	C
715	N→S	Hydrophilic	H
717	A→T	Hydrophobic→Hydrophilic	C

(H=helix, E=extended strand, C=random coil, T=turn)

Table 3: PROSITE motif assignments of PB2 protein of A/Indiana/1726/88(H1N1).

Site	Position	Motif
Casein kinase II	405 - 408	SqeD
phosphorylation site	489 - 492	SstE
	514 - 517	SpeE
	709 - 712	SinE
	717 - 720	**TkgE**
cAMP- and cGMP-dependent protein kinase phosphorylation site	663 - 666	KRlT
Protein kinase C	**471 - 473**	**TeK**
phosphorylation site	474 - 476	SmR
	491 - 493	TeR
	524 - 526	TeK
	559 - 561	TvK
	661 - 663	**StK**
	662 - 664	TkR
	751 - 753	TkR
N-myristoylation site	477 - 482	GlriSK
	590 - 595	GQysGF
	608 - 613	GTfdTV
	684 - 689	**GGveSA**
	685 - 690	GVesAV
Cell attachment sequence	415 - 417	RGD
N-glycosylation site	659 - 662	NKST

In strain A/Italy/671/1987, mutation T446F led a change from hydrophilic to hydrophobic amino acid and mutation V627K involved change of hydrophobic residue. In A/AUS/749/80 mutation I559T involved change of hydrophilic amino acid. In A/Afghanistan/1207/06 T451I mutation, tryptophan that is hydrophilic amino acid was replaced by hydrophobic isoleucine and T676N mutation changed hydrophobic amino acid.

Motif Search

Different motifs that were found in H1N1 are given in Table 3. Four new motifs were identified in A/swine/Indiana/1726/1988(H1N1). Mutations M473K (TeK) and 661 (StK) are reported at *Protein kinase C phosphorylation* site. Residues from 471 to 473 and 661 to 663 code for *Protein kinase C phosphorylation* motif (TeK). Mutation 684 is reported at *N-myristoylation* site and 717 at *Casein kinase II phosphorylation* site. Residues from 684 to 689 code for *N-myristoylation* motif (GGveSA) and residues from 717 to 720 code for *Casein kinase II phosphorylation* motif (TkgE). The most important finding noted was in *N-myristoylation* motif (GGveSA) where both the secondary structure and chemical property get changed (Table 2).

Discussion

PB2 protein is 759 amino acids long segment on the influenza A genome. This subunit binds to the 5'methyelated cap of pre-mRNA of host cells, before they cleave to provide primers for viral mRNA synthesis. Previous studies have shown that a monoclonal antibody recognizing an N-terminus proximal epitope of the PB2 [32] and polyclonal antibodies to the C-terminal region (amino acids 585–759) [33] inhibited viral genome transcription. These antibodies inhibited transcription by interfering with the process of primer recognition [32] or by blocking the endonucleolytic cleavage of a capped RNA primer [33, 34]. Blok et al. [33] have reported that polyclonal antibodies to the C-terminal region (amino acids 585–759) of the PB2 protein inhibited mRNA-primed transcriptions more effectively. In addition, Masunaga et al. [35] reported that polyclonal antibodies against the PB2 N-terminal amino acids 1–259, 206–459, or C-terminal 506–659 inhibited primer-extension activity. Our analyses showed that mutation (A684G) was identified in C-terminal region of PB2 of A/swine/Indiana/1726/1988 (H1N1) isolate which might make this isolate more resistive. The most important finding observed was identification of a new *N-myristoylation* motif found at position 684-689 in A/swine/Indiana/1726/1988 (H1N1). It is post translation motif, plays a vital role in membrane targeting and signal transduction. It is found in higher eukaryotes, fungi and viruses which are formed as a result of post-translation protein modification [36]. Myristoylation influences the conformational stability of individual proteins. Moreover, it also interacts with membranes or the hydrophobic motifs of other proteins [37]. This study also revealed that the change in secondary structure occurred, is due to mutation at 684 residue of this motif.

Conclusion

The PB2 subunit plays an important role in transcription regulation in viral mRNA synthesis. The study shows mutational difference of PB2 in different strains of H1N1 and H5N1. Mutations are seen in the development of new motifs of PB2. These findings have implications in understating PB2 function in viral multiplication and infection.

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