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Ebola Virus and Protein Engineering

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Abstract: Introduction: *The VP35 protein in Ebola virus is one of the main pathogenic components interacting to host cells and interferes with natural cellular immune responses. This is done by antagonistic potential of the viral VP35 protein which inhibits interferon regulatory factor 3 (IRF-3) protein in the infected cells and then induction of antiviral genes expression is disrupted. In the present in silico study, we have designed a novel recombinant IFNα/ß receptor protein which can be secreted out by host cells and circulate in blood stream.*
Methods: *the amino acid sequence of IFNα/ß was taken from NCBI, to design the recombinant IFNα/ß protein, functional, biophysical properties of VP35, and conserved domains were identified by Pfam and ProDom website. Few cystein amino acids were added to the adjacent region of identified domains to form disulfide bonds with possible cysteins present in VP35 polypeptide chains. The hydrophobicity index and net charge of the protein were studies by GPMAW and PepDraw website. For available signal peptide sequence are predicted by SignalP website and also conserved regions are identified by ClustalO. Next, to investigate the function of recombinant protein, the gene network involved in protein features functional pathways was identified by STRING and UCSF software.*
Result: *When the body encounters the virus, the recombinant protein can bind to VP35 and inhibit its antagonistic effect on IRF-3 proteins. We found instability index (57.47), Aliphatic index (85.50), Hydrophobicity index o (GPMAW as -0.276 Kcal/mol).*
Conclusion: *Evaluations made by bioinformatics software's revealed that the novel recombinant protein show promise for ameliorating the virulence of Ebola. But the obtained results should be investigated in vitro and in vivo for further evaluation and possible approval as a therapeutic strategy. These investigations are carried out to suggest a novel and efficient therapeutic approach for treatment of Ebola virus-mediated disease condition.*

Keywords: *IFNα/ß receptor, Ebola, VP35 protein, in silico design*

INTRODUCTION

Ebola virus (figure 1) belongs to Filoviridae family and is associated with hemorrhagic fever in human and primates. (Johnson, 1977 and Khan, 1999) It has a negative single stranded RNA of 19 kb as its genome. The viral genome encodes eight different proteins, (Sanchez et al.,1993), (Volchkov et al., 1995) which include nucleoprotein, glycoprotein, and soluble glycoprotein, VP24, VP30, VP35, VP40 and a RNA-dependent RNA polymerase. The VP34 is a crucial element in induction of disease condition. (Volchkov et al., 1995 and Sanchez et al, 1996) The VP35 protein interferes with natural resistance of host cells and makes the infection to spread in the body.

It has been shown that VP35 acts as an antagonist for interferon.(Basler et al, 2000) The protein may facilitate viral replication within infected cells by means of suppression of interferon-mediated immune responses.[7]

Interferons are a family of cytokines that are produced in response to viral infection. They act as antiviral agents, cellular growth inhibitors, and immune system regulators. (Sen, 2001), (Stark et al., 1998) In general, VP35 inhibits activation of interferon regulatory factor 3 (IRF-3), which is a main mediator in cascade of signal transduction in primary cellular response to viral infection. (Basler et al., 2003) During first steps of viral infection, the IRF-3 is activated in presence of dsRNA molecule and interacts with transcription factors regulating the early antiviral genes such as IFNα/β, IL-15, ISG15 and ISG56. (Grandvaux et al., 1989)

At present, by considering discovery of VP35 key structure, the necessary information can be obtained towards development of novel drugs. Since protein function is highly dependent to its spatial structure, it can be served as a new scope for disease treatment by protein 3D structure-based drug designing.

Methodology

In the present study, the amino acid sequence of IFNα/β was taken from NCBI database and then was virtually analyzed using bioinformatics software's. To design the recombinant IFNα/β protein, functional and conserved domains were identified by Pfam and ProDom website. Few cystein amino acids were added to the adjacent region of identified domains to form disulfide bonds with possible Cysteine present in VP35 polypeptide chains. By making use of ProtParm website some biophysical properties of VP35 protein such as halflife, instability index, and aliphatic index were measures and then were improved in the recombinant version of the protein.

The hydrophobicity index and net charge of the protein were studies by GPMAW and PepDraw website, respectively and then were subjected to some changes during construction of recombinant protein. According to the available signal peptide sequence of few secretory proteins which are predicted by SignalP website and their conserved regions are identified by ClustalO website, a signal peptide in order to secretion of protein to the outside of cell was design and replaced by the signal peptide that directs the protein to the cell membrane.

Next, to investigate the function of recombinant protein, the gene network involved in protein features functional pathways was identified by STRING website and then was compared to that of the normal protein. In addition, the amino acid sequence of recombinant protein was introduced to Pyre2 software to predict its 3D structure and then UCSF software was employed to draw the protein structure. Finally, the website, Reverse Translate, was undertaken to recognize the nucleotide sequence of the recombinant protein.

Results

Pfam has recognized two main domains that are located in 8-121 and 132-231 positions. Based on ProtParam prediction, the half-life of IFNα/β protein was 1.9 hours within mammalian bodies. (Ciechanover et al., 1989) Whereas valine has the maximum halflife among (Table 1) a valine amino acid has been added to the starting point of IFN-α/β protein polypeptide in order to increase its half-life.

Table 1. A comparative presentation of amino acids half-life in different organisms.

Amino acid	Mammalian	Yeast	E. coli

Cys	1.2 hour	>20 hour	>10 hour
Gln	0.8 hour	10 min	>10 hour
Glu	1 hour	30 min	>10 hour
Gly	30 hour	>20 hour	>10 hour
His	3.5 hour	10 min	>10 hour
Ile	20 hour	30 min	>10 hour
Leu	5.5 hour	3 min	2 min
Lys	1.3 hour	3 min	2 min
Met	30 hour	>20 hour	>10 hour
Phe	1.1 hour	3 min	2 min
Pro	>20 hour	>20 hour	-
Ser	1.9 hour	>20 hour	>10 hour
Thr	7.2 hour	>20 hour	>10 hour
Trp	2.8 hour	3 min	2 min
Tyr	2.8 hour	10 min	2 min
Val	100 hour	>20 hour	>10 hour

The instability index (Ii) (Guruprasad et al., 1990) of the normal protein was found to be 57.47 (estimated by ProtParam). For stable proteins Ii must be <40 and therefore, by making dipeptide changes at i site, the instability index of recombinant protein was decreased to reach to the desired range of Ii (lower than 40). Ii was calculated with following formula:

II = (10/L) * Sum DIWV (x[i] x [i+1])

The aliphatic index (Ai) of IFNα/ß was estimated to be 85.50. The aliphatic index (Ai) is defined as relative volume occupied by aliphatic side chains of proteins, which represents peptide stability against denaturation at high temperature. This index was calculated with following formula.(Atsushi, 1980) Alanine, valine, isoleucine and leucine are the four amino acids play key roles in level of aliphatic index of a polypeptide. Therefore, to enhance the Ai of the newly designed IFNα/ß protein structure, the aforementioned amino acids were replaced by other amino acids in some positions. As a result, the Ai was improved to 87.66.

Aliphatic index = X (Ala) + a* X (Val) + b* (X(Ile) + X (Leu))

Hydrophobic interactions are the relationships exist between non-polar regions of two molecules. (Haider, 2010) Histidine, phenylalanine, tryptophan and tyrosine are the main amino acids involved in such interactions. The hydrophobicity is the required energy to one mole of protein from hydrophilic environment to a hydrophobic environment. The hydrophobicity index of IFNα/ß protein was estimated by GPMAW as -

0.276 Kcal/ mol that was increased markedly in recombinant IFNα/ß through applying some amino acid substitutions.

The net electric charge of IFNα/ß receptor protein and VP35 was estimated to be -47 and -2, respectively. The net charge of protein is determined by differences between total acidic amino acid content of polypeptide with negative charge (e.g. Asparagine and guanine) and total basic ones with positive charges (e.g. Argenine and Lysine). To add more positive charges to the modified IFNα/ß protein and to increase VP35 affinity for IFNα/ß, few argentine and lysine amino acids are substituted by aspartic acid and glutamic acid that accelerates the net charge of recombinant protein to -37.

The signal peptide of IFNα/ß guides the protein towards the cell membrane. Based on few human secretory proteins, a signal peptide was designed and introduced to the structure of recombinant IFNα/ß that secretes the protein to the extra cellular matrix (Table 2).

Table 2. The amino acid sequences of few signal peptides used as template for design of a secretory signal peptide

>gi|183364|gb|AAA52578.1| GM-CSF [Homo sapiens]	**MWLQSLLLLGTVACSIS**
>gi|306806|gb|AAA35933.1|gro protein[Homo sapiens]	MARAALSAAPSNPRLLRVALLLLLLVAAGRRAAG
>gi|10834984|ref|NP_000591.1| interleukin-6 precursor [Homo sapiens]	MNSFSTSAFGPVAFSLGLLLVLPAAFP
>gi|386828|gb|AAA59172.1| insulin [Homo sapiens]	MALWMRLLPLLALLALWGPDPAAA
>gi|15021381|gb|AAK77664.1| transferin [Homo sapiens]	MRLAVGALLVCAVLGLCLA
>gi|386783|gb|AAA88080.1| haptoglobin [Homo sapiens]	MSALGAVIALLLWGQLFA
>gi|185362|gb|AAA02914.1| IgG [Homo sapiens]	MDWTWRFLFVVAAATGVQS
>gi|225768|prf||1313184B alpha1 antitrypsin	LLLAGLCCLLPGRLA
>gi|13528972|gb|AAH05278.1| Glucagon [Homo sapiens]	MKSIYFVAGLFVMLVQGSWQ
>gi|4557485|ref|NP_000087.1| Ceruloplasmin precursor [Homo sapiens]	MKILILGIFLFLCSTPAWA
The designed signal peptide	**MNSFSTSAFGPVAFSLGLLLVLPAAFP**

Exocytosis of modified IFNα/ß leads to earlier exposure VP35 to the recombinant protein prior to its attachment to the cell membrane and interaction with normal IFNα/ß protein. As the designed protein is a recombinant protein its amino acid sequence is not been deposited in biological databases. By comparing the genes involved in function of normal and recombinant proteins, it was evident that both of the proteins share a common functional pathway. If the amino acid sequence of protein was not registered in STRING software data reservoir, it recognizes the most similar polypeptide sequence.

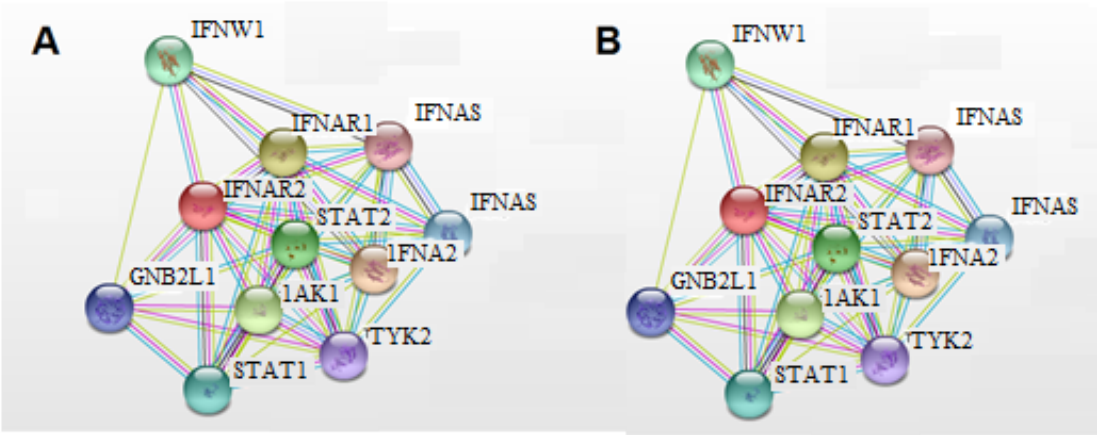

Fig1. The gene network of natural protein (A) and recombinant protein (B) by STRING software exhibit very similar functional pathway.

In case of our recombinant IFNα/ß protein, STRING analyses and 3-D structure comparisons made by Phyre2 and UCSF have revealed its enough homology with normal protein and showed that it could conserve its natural function (Fig 1 and Fig 2).

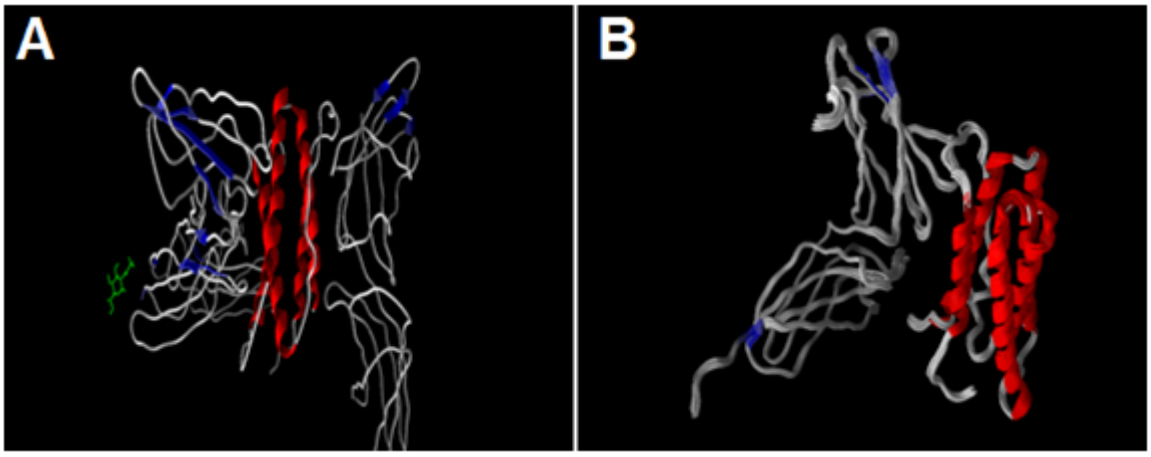

Fig 2. The 3-D structure of natural INF-α/ß (A) and recombinant protein (B) were drawn and analyzed by Phyre2 and UCSF. Finally DNA nucleotide sequence of the recombinant protein was identified by Reverse Translate software (Figure 4).

ATGAACAGCTTTAGCACCAGCGCGTTTGGCCCGGTGGCGTTTAGCCTGGGCCTGCTGCTGGTGCTGCCG
GCGGCGTTTCCGGTGTATGATTGCCCGGATTATACCGATGAAAGCTGCACCTTTAAAATTAGCCTGCGCA
ACTTTCGCAGCATTCTGAGCTGGGAACTGAAAAACCATAGCATTGTGCCGACCCATTATACCCTGCTGTA
TACCATTATGAGCAAACCGGAAGATCTGAAAGTGGTGAAAAACTGCGCGAACACCACCCGCAGCTTTTGC
GATCTGACCGATGAATGGCGCAGCACCCATGAAGCGTATGTGACCGTGCTGGAAGGCTTTAGCGGCAAC
ACCACCCTGTTTAGCTGCAGCCATAACTTTTGGCTGGCGATTGATATGAGCTTTGAACCGCCGGAATTTG

AAATTGTGGGCTTTACCAACCATATTAACGTGATGGTGTGCTTTCCGAGCATTGTGGAAGAAGAACTGCA
GTTTGATCTGAGCCTGGTGATTGAAGAACAGAGCGAAGGCATTGTGAAAAAACATAAACCGGAAATTAAA
GGCAACATGAGCGGCAACTTTACCTATATTATTGATAAACTGATTCCGAACACCAACTATTGCGTGAGCG
TGTATCTGGAACATAGCGATGAACAGGCGGTGATTAAAAGCCCGCTGAAATGCACCCTGCTGCCGCCGG
GCCAGGAAAGCGAAAGCGCGGAAAGCGCGAAAATTGGCGGCATTATTACCGTGTTTCTGATTGCGCTGG
TGCTGACCAGCACCATTGTGTGCCTGAAATGGATTGGCCATATTTGCCTGCGCAACAGCCTGCCGAAAGT
GCTGAACTTTCATAACTTTCTGGCGTGGCCGTTTCCGGCGCTGCCGCCGCTGGAAGCGATGGATATGGTG
GAAGTGATTTATATTAACCGCAAAGTGAAAGTGTGGGATTATAACTATGATTTTGAAAGCGATAGCAAAA
CCGAAGCGGCGCCGCGCACCTGGGGCGGCGGCTATACCATGCATGGCCTGACCGTGCGCCCGCTGGGCC
AGGCGAGCGCGACCAGCACCTGGAGCCAGCTGATTGATCCGCGCAGCGAAAAAGAACCGGATCTGCCGA
TTGTGGATGTGGAACTGACCACCATGCCGAAACGCAGCCCGCAGCTGCTGGAACTGCTGAGCCGCCCGT
GCGAACGCCGCAAAAGCCCGCTGCAGGATCCGTTTCCGGAAGAAGATGTGAGCAGCACCGAAGGCAGCG
GCGGCCGCATTACCTTTAACGTGGATCTGAACAGCGTGTTTCTGCGCGTGCTGGATGATGAAGATAGCGA
TGATCTGGAAGCGCCGCTGATGCTGAGCAGCCATCTGGAAGAAATGGTGGATCCGGAAGATCCGGATAA
CGTGCAGAGCCTGCATCTGCTGGCGAGCGGCGAAGGCACCCAGCCGACCTTTCCGAGCCCGAGCAGCGA
AGGCCTGTGGAGCGAAGATGCGCCGAGCGATCAGAGCGATACCAGCTTTAGCGATGTGGATCTGGGCGA
TGGCTATATTGTGCGC

Fig 3. DNA sequence of the modified INF-α/ß protein.

Taken together, the design poly peptide could function as the natural version of the same in many aspects. Moreover, following secretion to the blood stream it can be bind to the viral VP35 protein and inhibits its antagonistic action against IFR-3. That is how the recombinant protein allows the host cell to have their normal and efficient interleukin-mediated immune response when viral infection is met. The Glycosylation areas identified with Allerdicttor website in order to prevent the occurrence of autoimmune reaction in the natural protein.

Discussion

It is a well-known fact that protein function is highly dependent to its spatial structure. Minute changes in protein structure can cause severe impairments to its normal function. (Lehrman ,1990, Alberts et al. 2010) Characterization of protein structure opens a new window in order to predict the protein function. Modification in function of a specific protein can be achieved by implying desired structural changes. The hydrophobicity index was increased markedly in recombinant IFNα/ß through applying some amino acid substitutions. It was evident of normal and recombinant proteins that both of the proteins share a common functional pathway.

Conclusion

These investigations are carried out to suggest a novel and efficient therapeutic approach for treatment of Ebola virus-mediated disease condition. Nevertheless, *in vitro* and *in vivo* tests need to be performed in future to verify the potential of recombinant IFNα/ß in amelioration of Ebola virus virulence as an effective treatment strategy. In the present research work we have performed primary in silico studies to design a new

IFNα/ß protein which can be secreted to the external cellular matrix, bind to and block the Ebola VP35 protein.

Ethical issues

Not applicable

Competing interests

Authors declare no any problem conflict of interests

Research Highlights

What is current knowledge?

- ✓ In the present in silico study, we have designed a novel recombinant IFNα/ß receptor protein which can be secreted out by host cells and circulate in blood stream.
- ✓ Novel recombinant IFNα/ß receptor proteins which can be secreted out by host cells and circulate in blood stream.

What is new here?

- ✓ These investigations suggest a novel and efficient therapeutic approach for treatment of Ebola virus-mediated disease condition.

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