The Pharma Innovation Journal 2021; SP-10(3): 179-182

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(3): 179-182

www.thepharmajournal.com
Received: 11-01-2021
Accepted: 16-02-2021

Manikandan R
Immunology Section,
ICAR- Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Bindu S
Immunology Section,
ICAR- Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Dinesh M
Division of Pathology,
ICAR- Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Shanmuganathan S
Division of Veterinary Virology,
ICAR-Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Corresponding Author:
Manikandan R
Immunology Section,
ICAR- Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India

Computational and bio-informatics analysis of type I recombinant canine interferon-epsilon

Manikandan R, Bindu S, Dinesh M and Shanmuganathan S

Abstract
Interferons (IFNs) are the small cellular polypeptides with a broad range of biological activities like antiviral, anti-proliferative and immunodulation activity. In the canine population, type I IFNs have primarily been explored as efficient antiviral therapeutic agents against a wide range of viral diseases. Among the type I IFNs, recombinant INF-ε has potent antiviral activity in both homologous and heterologous cell lines against a wide range of viruses. Nevertheless, there is limited knowledge regarding the computational and bioinformatics analysis of the potent antiviral therapeutic agent, the recombinant canine IFN-ε (CaIFN-ε) protein. Several online bioinformatics software programs were used to analyze the characteristics of the potent CaIFN-ε protein, including the identification of potential O-glycosylation sites, N-glycosylation sites, signal peptide cleavage sites, phosphorylation sites and trans-membrane regions. Furthermore, we utilized online software to predict antigen epitopes, secondary and three-dimensional structures of the CaIFN-ε protein. Hence, this computational and bioinformatics analysis aids in getting deeper knowledge and further insights into the promising, effective therapeutic potential of recombinant CaINF-ε for treating different viral infections in canines.

Keywords: broilers, garlic, tulasi, bodyweight, feed conversion ratio, livability

1. Introduction
Interferons (IFNs) were first identified in the 1950s and characterized as a substance that interferes with the replication of vaccinia and influenza viruses (Isaacs and Lindenmann, 1957) [1]. IFNs are a group of cytokines that play an important role in the regulation and activation of both innate and adaptive immune responses (Meyer, 2009) [2]. They can be subdivided into type I (IFN-α, IFN-β, IFN-δ, IFN-ε, IFN- ζ, IFN-κ, IFN-τ and IFN-ω), type II (IFN-γ) and type III (IFN-λ) (Klotz *et al*., 2017) [3]. Canine IFN-epsilon (CaIFN-ε) was cloned and expressed in *Escherichia coli*, and the recombinant protein demonstrated high antiviral activity, anti-proliferation activity, and Natural Killer (NK) cell stimulatory activity (Yang *et al*., 2013) [4]. Recombinant canine IFN-epsilon (rCaIFN-ε) exhibited strong antiviral activity on both homologous and heterologous animal cells in *in vitro*, suggesting that recombinant CaIFN-ε has more broad cross-species activity than recombinant canine IFN-alpha (CaIFN-α) (Yang *et al*., 2013) [4]. This study provides a computational and bioinformatics research analysis of recombinant CaIFN-ε protein that can provide further insights to the promising antiviral therapeutic agent.

2. Materials and Methods
2.1 Sequence retrieval
The rCaIFN-ε sequence retrieved from the National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/) through the accession number (AGO15240.1).

2.2 Analysis of CaINF-ε characteristics
Several online bioinformatics software programs were used to analyze the characteristics of the CaIFN-ε protein: Signal peptide cleavage sites were analyzed by the SignalP 3.0 server at http://www.cbs.dtu.dk/services/SignalP-3.0/; phosphorylation sites were analyzed by the NetPhos3.1server at http://www.cbs.dtu.dk/services/NetPhos/; N-glycosylation sites were analyzed by the NetNGlyc1.0 server at http://www.cbs.dtu.dk/services/NetNGlyc/; O-glycosylation sites were analyzed by the YinOYang1.2 server at http://www.cbs.dtu.dk/services/YinOYang/; trans-membrane regions were analyzed by the TMHMM 2.0 server at http://www.cbs.dtu.dk/services/TMHMM/; antigen epitopes and hydrophobicity were analyzed by the BepiPred 1.0 server at

http://www.cbs.dtu.dk/services/BepiPred-1.0/; Secondary and three-dimensional structures were predicted by SOPMA at https://npsaprabi.ibcp.fr/cgibin/npsa_automat.pl?page=/NPSA/npsa_sopma.html and the three-dimensional structures of canine IFN-ε predicted with SWISS-MODEL software (Wang *et al*., 2020) [5].

3. Results

3.1 Sequence retrieved

CaINF-ε protein sequence retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/) through the accession number (AGO15240.1).

3.2 Bioinformatics analysis for CaINF-ε

The properties of the CaINF-ε were assessed using several online bioinformatics software programs, including the detection of potential N-glycosylation sites, phosphorylation sites, O-glycosylation sites, signal peptide cleavage sites, subcellular localization and trans-membrane regions.

3.2.1 Glycosylation sites

There is two N-glycosylation potential site at 94 and 184 aa residue (Figure: 1) and 5 O-glycosylation potential sites at 47, 65, 131, 161 and 186 aa residues (Figure: 2) were predicted in CaINF-ε.

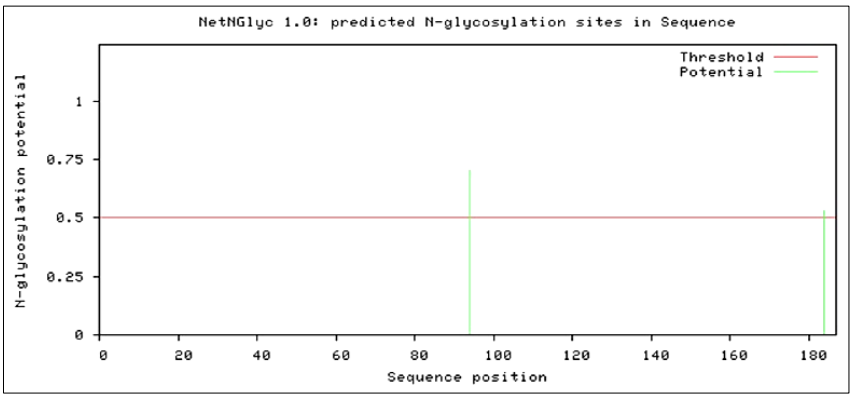

Fig 1: Prediction of N-glycosylation potential sites in CaINF-ε. The green color line which reaches above the red color threshold level is the predicted N-glycosylation potential sites (X-axis: Sequence position, Y-axis: N-glycosylation potential)

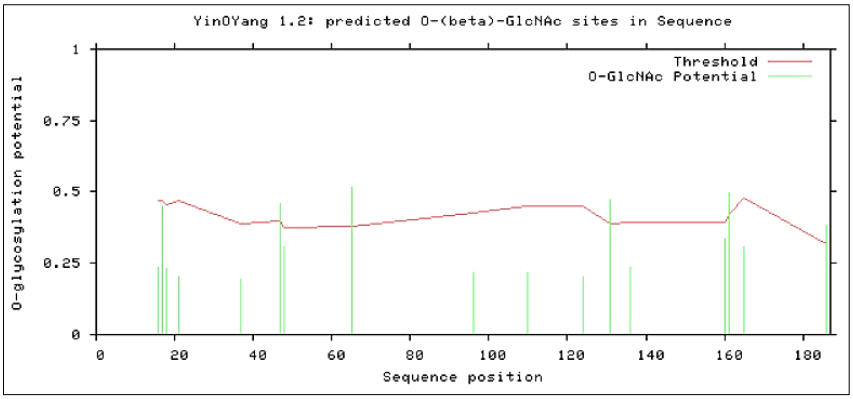

Fig 2: Prediction of O-glycosylation potential sites in canine INF-ε. The green color line which reaches above the red color threshold level is the predicted O-glycosylation potential sites (X-axis: Sequence position, Y-axis: O-glycosylation potential)

3.2.2 Phosphorylation and signal peptide cleavage sites

There are totally 9 Phosphorylation sites (serine-9) (Figure: 3) were predicted and most likely signal peptide cleavage site was predicted between aa position 21 and 22 (Ala-His) (Figure: 4).

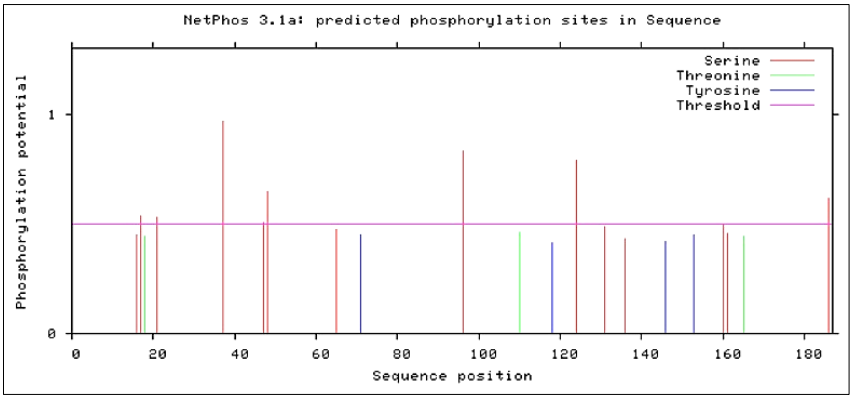

Fig 3: Prediction of potential phosphorylation sites (Serine-9) in CaINF-ε (X-axis: Sequence position, Y-axis: O-glycosylation potential)

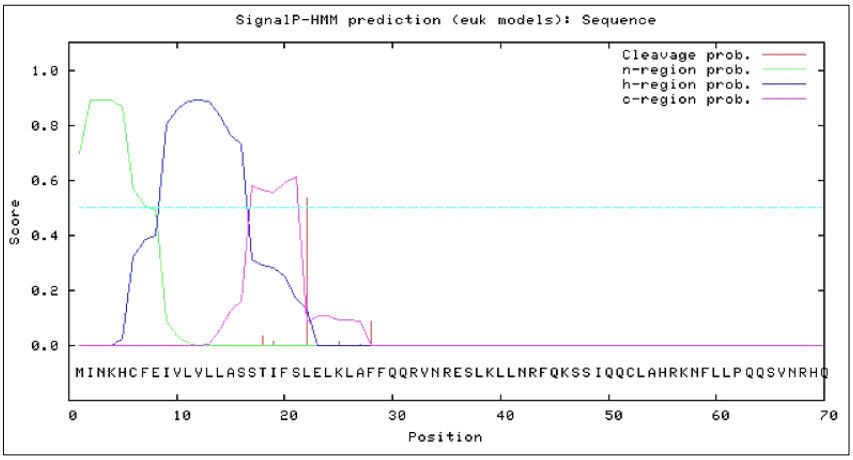

Fig 4: Prediction of potential signal peptide cleavage sites in CaINF-ε. The most likely predicted signal cleavage was between aa residue IFS-LE (Ser21-Leu22) (X-axis: Sequence position, Y-axis: Score)

3.2.3 Antigen epitopes and trans-membrane region

The antigenic epitopes of CaINF-ε (predicted by BepiPred 1.0 software) were located at amino acids residues 65-73, 100, 102, 103, 127-136, 138-141 and 156-160. The predicted trans-membrane region for CaINF-ε is located at inside 1-6 residues, TM helix 7-29 residues and outside 30-187 residues.

3.2.4 Secondary and three-dimensional structure

SOPMA software was used to predict the secondary structure, that revealed CaINF-ε contained 66.31% alpha helix, 3.21% beta sheet, 2.14% turn and 28.34% irregular coil structures (Figure 5) and the three-dimensional structures of CaINF-ε was predicted with SWISS-MODEL software as shown in Figure 6.

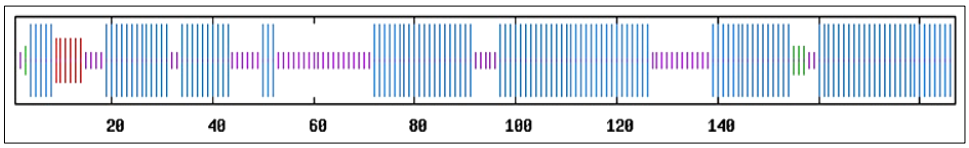

Fig 5: The predicted secondary structure of CaINF-ε

Fig 6: The predicted three-dimensional structure of CaINF-ε

4. Discussion

Human IFN-ε was first identified in 1999 (Chen *et al*., 1999), that has found to be constitutively expressed in the lung, brain, small intestine, and reproductive tissues (Hardy *et al*., 2004; Peng *et al*., 2007) [7, 8]. Since then, mice, pigs, cattle and canine IFN-ε have been reported (Hardy *et al*., 2004; Yulei *et al*., 2008; Walker and Roberts, 2009; Yang *et al*., 2013) [4, 7, 9, 10].

Recombinant CaIFN-ε and CaIFN-α7 were tested for antiviral activities against Vesicular stomatitis virus (VSV), Canine parvovirus-2 (CPV-2), Canine distemper virus (CDV), and H1N1 in the MDCK cells and both exhibited activity against these viruses. CaIFN-α7 was more active against VSV and CPV-2 than CaIFN-ε, whereas CaIFN-ε was more active against CDV and H1N1. CaIFN-α7 was most active against VSV and least active against H1N1, while CaIFN-ε was most and least active against VSV and CPV-2, respectively. In addition, CaIFN-ε exhibited a significant anti-proliferative response against A72 canine tumor cells and MDCK canine epithelial cells in a dose-dependent manner and activates the JAK-STAT signaling pathway (Yang *et al*., 2013) [4]. The antiviral activity of recombinant CaIFN-ε is reported against VSV in both heterologous cell lines of MDBK and HeLa cell lines (Klotz *et al*., 2017) [3].

Glycosylation is a post-translational modification process that affects the antigenic determinants, enzymatic properties, charge characteristics, and thermal stability of proteins. Studies have reported that glycosylation sites can play a crucial role in determining the activity of IFNs (Buckwold *et al*., 2007) [11]. Glycosylated IFN-ω has been shown to be significantly more potent than non-glycosylated IFN-ω against hepatitis C virus, yellow fever virus, BVDV and West Nile virus, with even more superior than IFN-α, IFN-β and IFN-γ (Li *et al*., 2017) [12]. However, CaIFN-ε was predicted with 2 N-glycosylation site and 5 O-glycosylation sites by using online bioinformatics software programs.

The mature proteins with normal biological activity can be formed, only after the signal peptide sequence was removed from the precursor protein, thus allowing them to be secreted outside the cell membrane (Lu *et al*., 2020) [13]. We used online software to predict the signal peptide sequence of the CaIFN-ε protein consists of 21 amino acid residues, and that

the signal peptide cleavage site is located between residues Ser21-Leu22. The results indicated that the rCaIFN-ε protein could be expressed in vitro in their soluble forms. In addition, CaIFN-ε protein was predicted with 9 phosphorylation sites and trans-membrane region for CaINF-ε is located at inside 1-6 residues, TM helix 7-29 residues and outside 30-187 residues.

Several online bioinformatics software programs were used to analyze novel feline IFN-ωa (feIFN-ωa) protein, including the identification of potential signal peptide cleavage sites in Gly23-Cys24, 0 N-glycosylation sites, 9 O-glycosylation sites, 15 phosphorylation sites, intercellular trans-membrane regions and for feline IFN-ωb (feIFN-ωb), signal peptide cleavage sites in Gly23-Cys24, 0 N-glycosylation sites, 6 O-glycosylation sites, 13 phosphorylation sites, intercellular trans-membrane regions (Wang *et al*., 2020) [5].

The antigen epitopes of CaIFN-ε (predicted by BepiPred 1.0 software) are located at amino acids residues 65-73, 100, 102, 103, 127-136, 138-141 and 156-160. While the antigen epitopes of feIFN-ωa are located at amino acids residues 19-23, 27-34, 66, 70-74, 96-107, 128-138, 158-162, 179-182, and 192-186 and the antigen epitopes of feIFN-ωb were located at amino acid residues 19-23, 27-33, 70-76, 96-107, 128-145, 165-169 and 200-203 (Wang *et al*., 2020) [5].

According to the previous reports of secondary structure prediction in feIFN-ωa using SOPMA software revealed 62.24% alpha helix, 2.55% beta sheet, and 34.18% irregular curl structures, whereas feIFN-ωb contained 65.52% alpha helix, 1.97% beta sheet, and 31.53% irregular curl structures (Wang *et al*., 2020) [5]. However, secondary structure of the CaIFN-ε protein was predicted using SOPMA software revealed as 66.31% alpha helix, 3.21% beta sheet, 2.14% turn and 28.34% irregular coil structures. The three-dimensional structures of CaIFN-ε were predicted with SWISS-MODEL software as shown in figure 6. Along with a broad cross-species antiviral activity against various viruses in both homologous and heterologous cell lines, this bioinformatics study analysis may provide a better knowledge and further insights into the therapeutic potential of recombinant CaIFN-ε.

5. Conclusion

The biological functions of recombinant CaIFN-ε, including antiviral, anti-proliferative, and NK cytotoxicity promoting functions indicated that IFN-ε is a likely candidate for a novel, effective therapeutic agent. The signal peptide sequences, phosphorylation sites, glycosylation sites, antigen epitopes and trans-membrane regions of the rCaINF-ε protein were analyzed using bioinformatics to provide better theoretical guidance for the functional study of the protein. Recombinant CaIFN-ε displayed potent antiviral activity on both homologous and heterologous animal cells in vitro, indicating broad cross-species activity and might be candidate for the development of useful therapeutic medicines to treat viral infections in pet animals.

6. References

1. Isaacs A, Lindenmann J, Valentine RC. Virus interference. II. Some properties of interferon. Proceedings of the Royal Society of London. Series B-Biological Sciences 1957;147(927):268-273.
2. Meyer O. Interferons and autoimmune disorders. Joint Bone Spine 2009;76(5):464-73.
   doi: 10.1016/j.jbspin.2009.03.012. Epub 2009 Sep 20. PMID: 19773191.
3. Klotz D, Baumgärtner W, Gerhauser I. Type I interferons in the pathogenesis and treatment of canine diseases. Veterinary immunology and immunopathology 2017;191:80-93.
4. Yang L, Xu L, Li Y, Li J, Bi Y, Liu W. Molecular and functional characterization of canine interferon-epsilon. Journal of Interferon & Cytokine Research 2013;33(12):760-768.
5. Wang X, Li F, Han M, Jia S, Wang L, Qiao X *et al*. Cloning, prokaryotic soluble expression, and analysis of antiviral activity of two novel feline IFN-ω proteins. Viruses 2020;12(3):335.
6. Chen J, Godowski P, Wood WI, Zhang DX. Genentech Inc. Human interferon-epsilon: a type I interferon. U.S. Patent 2001;6:299, 869.
7. Hardy MP, Owczarek CM, Jermiin LS, Ejdebäck M, Hertzog PJ. Characterization of the type I interferon locus and identification of novel genes. Genomics 2004;84(2):331-345.
8. Peng FW, Duan ZJ, Zheng LS, Xie ZP, Gao HC, Zhang H *et al*. Purification of recombinant human interferon-ε and oligonucleotide microarray analysis of interferon-ε-regulated genes. Protein expression and purification 2007;53(2):356-362.
9. Yulei T, Yanling W, Weijie W, Liqiang H, Zhiqiang Z, Meng Z *et al*. Clone and Construction of Recombinant Expression Vector of Porcine IFNE1. Chin Agric Sci Bull 2008;11:60-64.
10. Walker AM, Roberts RM. Characterization of the bovine type I IFN locus: rearrangements, expansions, and novel subfamilies. BMC genomics 2009;10(1):1-15.
11. Buckwold VE, Wei J, Huang Z, Huang C, Nalca A, Wells J *et al*. Antiviral activity of CHO-SS cell-derived human omega interferon and other human interferons against HCV RNA replicons and related viruses. Antiviral research 2007;73(2):118-125.
12. Li SF, Zhao FR, Shao JJ, Xie YL, Chang HY, Zhang YG. Interferon-omega: Current status in clinical applications. International immunopharmacology 2017;52:253-260.
13. Lu X, Zhong Q, Liu J, Yang F, Lu C, Xiong H *et al*. Efficient expression of chondroitinase ABC I for specific disaccharides detection of chondroitin sulfate. International journal of biological macromolecules 2020;143:41-48.