The Pharma Innovation Journal 2021; SP-10(3): 44-48

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2021; SP-10(3): 44-48

www.thepharmajournal.com
Received: 11-01-2021
Accepted: 26-02-2021

Manikandan R
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 bioinformatics analysis of type I recombinant canine interferon-alpha 7: A potential anti-viral therapeutic agent

Manikandan R, Dinesh M and Shanmuganathan S

Abstract
Interferons (IFNs) are representative cytokines that play important roles in innate immunity, which is produced to defend against pathogens. Interferons (IFNs), especially type I IFNs (INF-α and INF-β) have been explored as effective antiviral therapeutic drugs against a wide range of viral diseases in the canine population. Among the type I IFN-α subtypes, recombinant INF-α7 has potent antiviral activity in homologous 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 canine recombinant IFN-α7 (CaIFN-α7) protein. Several online bioinformatics software programs were used to analyze the characteristics of the potent CaIFN-α7 protein, including the identification of potential N-glycosylation sites, O-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-α7 protein. Hence, this computational and bioinformatics analysis aids in getting deeper knowledge and further insights into the promising, effective therapeutic potential of CaINF-α7 for treating different viral infections in canines.

Keywords: bioinformatics, type I recombinant canine interferon-alpha 7, anti-viral therapeutic

1. Introduction
In 1957, soluble glycoproteins with strong antiviral activity were initially identified as interferons (IFNs) (Isaacs *et al*., 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]. Biological activity of IFNs includes anti-proliferative, anti-viral and immunomodulatory effects in the host immune response to viral or bacterial infection. Types I, II, and III of IFNs have been categorized based on their genetic, structural, and functional features, along with their cell-surface receptors (Zhou *et al*., 2014) [3]. The dog genome has 10 functional IFN- α genes and 2 truncated pseudogenes, four of which are clustered with canine IFN-ε, IFN-κ, and IFN-β on chromosome 11 (Yang *et al*., 2013) [4]. However, the specific sequences, locations, and names of the different CaINF-α subtypes have not been reported and only 5 different IFN-α subtypes has been identified in the dog genome based on published sequences (Klotz *et al*., 2017) [6]. The antiviral activity of recombinant canine INF-alpha 7 (CaIFN-α7) was more potent than that of the other CaINF-α subtypes (Taira *et al*., 2005) [5]. CaINF-α7 displayed antiviral activities against a wide range of viruses namely; canine adenovirus (CAV-1), canine distemper virus (CDV), canine herpes virus (CHV), canine parvovirus (CPV-2), influenza A virus (IAV) in MDCK cells (Klotz *et al*., 2017) [6]. This study provides a bioinformatics analysis of recombinant canine INF-α7 protein that can provide further insights.

2. Materials and Methods
2.1 Sequence retrival
The recombinant CaINF-α7 protein sequence retrieved from the National Center of Biotechnology Information (https://www.ncbi.nlm.nih.gov/) through the accession number (NP_001006655.1).

2.2 Analysis of CaINF-α7 characteristics
Several online bioinformatics software programs were used to analyze the characteristics of the CaIFN-α7 protein: Signal peptide cleavage sites were analyzed by the Signal P3.0 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-α7 predicted with SWISS-MODEL software (Wang *et al*., 2020).

3. Results

3.1 Sequence retrieved

CaINF-α7 protein sequence retrieved from the NCBI (https://www.ncbi.nlm.nih.gov/) through the accession number (NP_001006655.1).

3.2 Bioinformatics analysis for CaINF-α7

The properties of the CaINF-α7 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 one N-glycosylation potential site at 101 aa residue (Figure: 1) and 4 O-glycosylation potential sites at 6, 8, 95 and 96 aa residues (Figure: 2) were predicted in CaINF-α7.

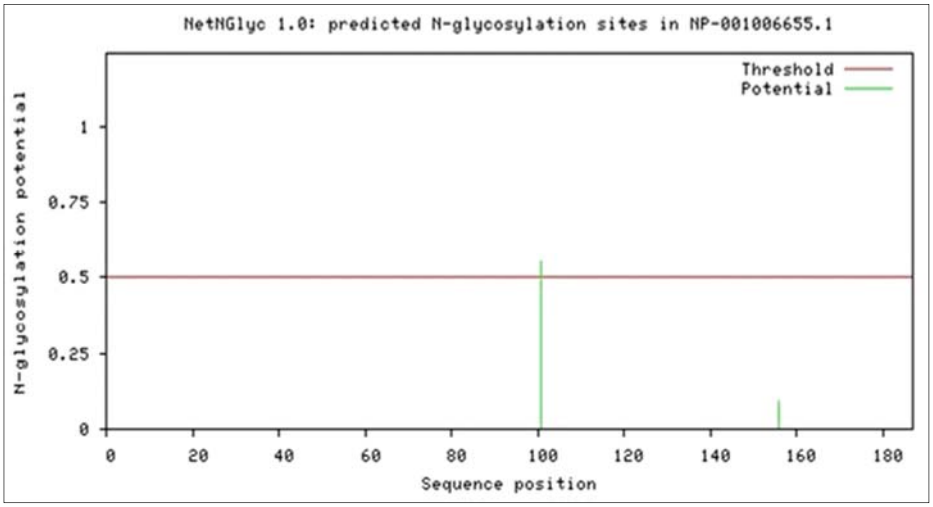

Fig 1: Prediction of N-glycosylation potential sites in CaINF-α7. The green color line which reaches above the red color threshold level is the predicted N-glycosylation potential sites (X-axis: Sequence position, Y-axis: N-glycosylation potential).

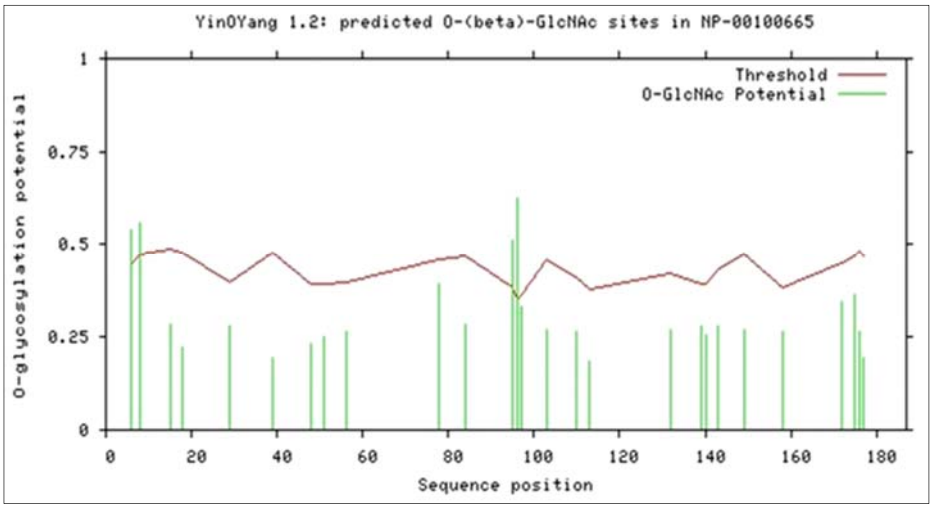

Fig 2: Prediction of O-glycosylation potential sites in canine INF-α7. The green color line which reaches above the red color threshold level is the predicted O-glycosylation potential sites (X-axis: Sequence position, Y-axis: O-glycosylation potential).

3.2.2 Phosphorylation and signal peptide cleavage sites

There are totally 16 Phosphorylation sites (serine-9, Theroine-6, Tyrosine-1) (Figure: 3) were predicted and most likely signal peptide cleavage site was predicted between aa position 23 and 24 (Ala-His) (Figure: 4).

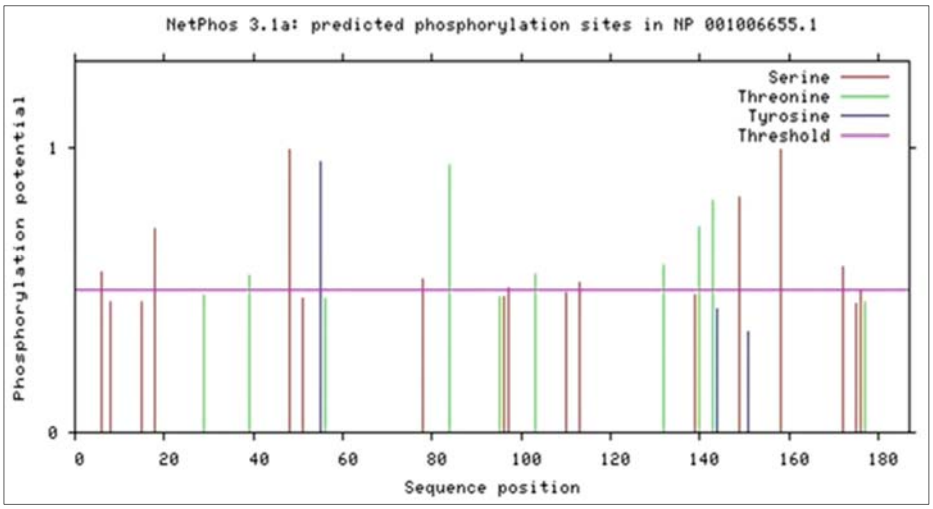

Fig 3: Prediction of potential phosphorylation sites (Serine-9, Theroine-6, and Tyrosine-1) in CaINF-α7 (X-axis: Sequence position, Y-axis: O-glycosylation potential).

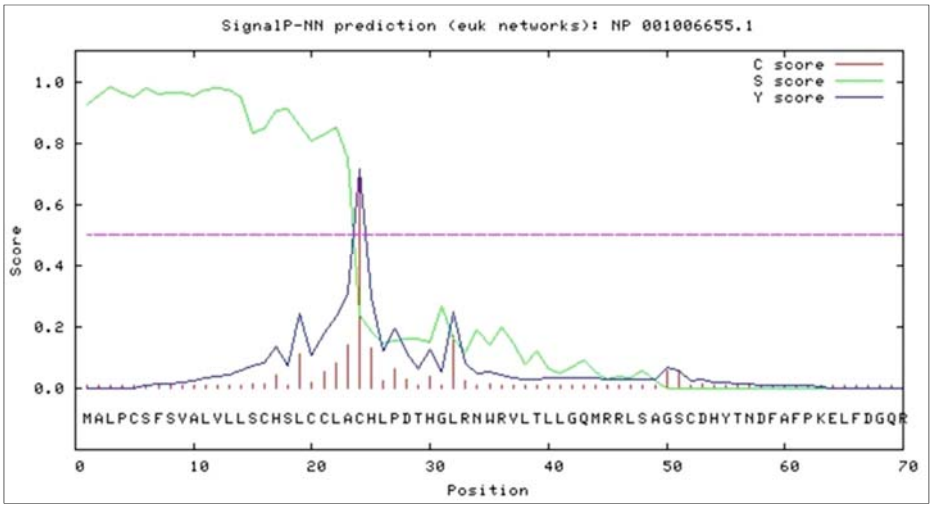

Fig 4: Prediction of potential signal peptide cleavage sites in CaINF-α7. The most likely predicted signal cleavage was between aa residue CLA-CH (Ala23-Cys24). (X-axis: Sequence position, Y-axis: Score).

3.2.3 Antigen epitopes

The antigenic epitopes of CaINF-α7 (predicted by BepiPred 1.0 software) were located at amino acids residues 30-31, 52-56, 60, 66, 70-72, 94-99, 114, 117, 124, 127-130, 134-136, and 155-159.

3.2.4 Secondary and three-dimensional structure

SOPMA software was used to predict the secondary structure, that revealed CaINF-α7 contained 61.50% alpha helix, 3.74% beta sheet, 3.74% turn and 31.02% irregular coil structures (Figure 5) and the three-dimensional structures of CaINF-α7 was predicted with SWISS-MODEL software as shown in Figure 6.

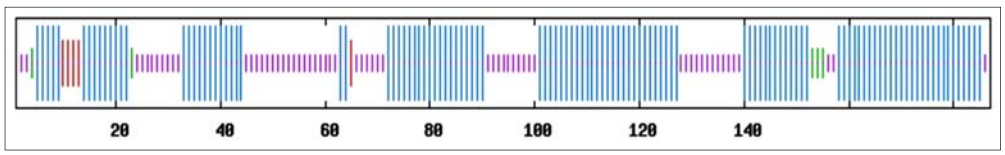

Fig 5: The predicted secondary structure of CaINF-α7

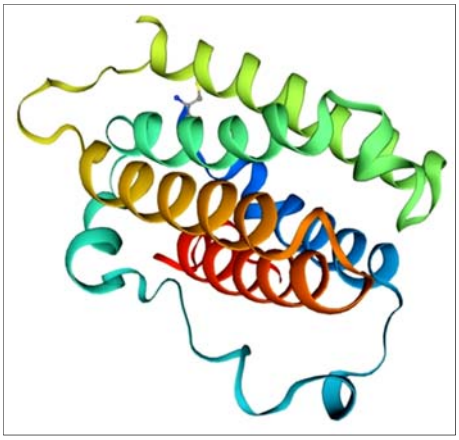

Fig 6: The predicted three-dimensional structure of CaINF-α7

4. Discussion

Recombinant CaIFN-α7 and CaIFN-α8 exhibited antiviral activity on three types of cells: MDCK, A-72, and Cf2Th, but only on heterologous cells. The Antiviral effects of rCaIFN-α7 and rCaIFN-α8 against vesicular stomatitis virus (VSV) and CAV-1 were detected, but not aginst CHV-1. rCaIFN-α7 was 4-5 fold more potent than rCaIFN-α8 in terms of its antiviral activities (Taira *et al*., 2005) [5]. Recombinant CaIFN-ε and CaIFN-α7 were tested for antiviral activities against VSV, CPV-2, 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 (Yang *et al*., 2013) [4].

Glycosylation is a post-translational modification process that affects the antigenic determinants, charge characteristics, enzymatic properties, 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) [10]. However, CaIFN-α7 was predicted with 1 N-glycosylation site and 4 O-glycosylation sites by using online bioinformatics software programs.

The mature proteins, with normal biological activity, were 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) [11]. We used online software to predict the signal peptide sequence of the CaIFN-α7 protein consists of 23 amino acid residues, and that the signal peptide cleavage site is located between residues Ala23-Cys24.The results indicated that the recombinant rCaIFN-α7 protein could be expressed in vitro in their soluble forms. In addition, CaIFN-α7 protein was predicted with 16 phosphorylation sites and presence of intracellular transmembrane regions.

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) [7]. 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- B and IFN- γ (Li *et al*., 2017) [12].

The antigen epitopes of CaIFN-α7 (predicted by BepiPred 1.0 software) are located at amino acids residues 30-31, 52-56, 60, 66, 70-72, 94-99, 114, 117, 124, 127-130, 134-136, and 155-159. 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) [7].

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) [7]. However, secondary structure of the CaIFN-α7 protein was predicted using SOPMA software revealed as 61.50% alpha helix, 3.74% beta sheet, 3.74% turn and 31.02% irregular coil structures. The three-dimensional structures of CaIFN-α7 were predicted with SWISS-MODEL software as shown in figure 6. Along with a broad spectrum of antiviral activity against various viruses, bioinformatics study analysis may provide a better knowledge and further insights into the therapeutic potential of canine INF-α7.

5. Conclusion

The combination of antiviral therapy has become a common practice in treating canine viral infection due to pharmacokinetics and the short half-life of IFN alone. The signal peptide sequences, signal peptide cleavage sites, phosphorylation sites, glycosylation sites, antigen epitopes and trans-membrane regions of the recombinant CaINF-α7 protein were analyzed using bioinformatics to provide better theoretical guidance for the functional study of the protein. The antiviral activity of recombinant CaINF-α7 was more potent than that of the other canine recombinant INF-α subtype, indicating that they might be candidates 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. Zhou H, Chen S, Wang M, Cheng A. Interferons and their receptors in birds: a comparison of gene structure, phylogenetic analysis, and cross modulation. International journal of molecular sciences 2014;15(11):21045-21068.
4. Yang L, Xu L, Li Y, Li J, Bi Y, Liu W. Molecular and functional characterization of canine interferon-epsilon. J Interferon Cytokine Res 2013;33:760-768.
5. Taira O, Watanugi I, Hagiwara Y, Takahashi M, Arai S, Sato H *et al*. Cloning and expression of canine interferon-α genes in *Escherichia coli*. Journal of veterinary medical science 2005;67(10):1059-1062.
6. 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.
7. 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.
8. Taira O, Watanugi I, Hagiwara Y, Takahashi M, Arai S, Sato H *et al*. Cloning and expression of canine interferon-α genes in Escherichia coli. Journal of veterinary medical science 2005;67(10):1059-1062.
9. 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.
10. 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.
11. 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.
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.