

# Molecular interactions between *Bos taurus* interferon- $\tau$ 1c and human type I interferon receptor

Vishawdeep Singh Jamwal, Gourav Modi, Aman George\*, Manmohan Singh Chauhan

Embryo Biotechnology Lab, Animal Biotechnology Centre, National Dairy Research Institute, Karnal-132001, Haryana, India;

\*Present Address: Shri Mata Vaishno Devi University-182320, J&K, India; Aman George – E-mail: george.emb.bio@gmail.com;

\*Corresponding author

Received June 28, 2009; Revised August 17, 2009; Accepted September 11, 2009; Published October 13, 2009

## Abstract:

Interferon (IFN)- $\tau$  secreted only by ruminant endometrium, helps in maternal recognition of pregnancy and exhibit antiviral and antiproliferative activity. Among different types of IFN- $\tau$ , IFN- $\tau$ 1c and IFN- $\tau$ 3a are the most highly expressed isoforms. In the present study structure of INF- $\tau$ 1c was predicted using homology modelling. The best model was selected based on overall stereo-chemical quality. The generated 3D structure of the Interferon- $\tau$ 1c protein of *Bos taurus* was predicted using the ovine interferon- $\tau$  (PDB ID: 1B5L\_A) as template. The structure comprises of 5  $\alpha$  helices separated by loop regions, which is similar to the one predicted for other IFNs. Molecular interactions of bovine IFN- $\tau$ 1c with human interferon Type 1 receptor (IFNAR1) was explored in an attempt to predict human IFNAR1 binding sites of IFN- $\tau$ 1c.

**Keywords:** interferon; interactions; human interferon receptor; *Bos taurus*

## Background:

The Interferon (IFN)- $\tau$  a member of Type I IFN, produced by ruminant ungulates, during blastocyst stage and the time when the elongated trophoblast attaches to the uterine wall, prevents the destruction of the corpus luteum and helps in maternal recognition of pregnancy. IFN- $\tau$  acts on the endometrium to abrogate production of luteolytic pulses of PGF $2\alpha$ , produced from the uterine endometrium [1-4] by reducing expression of estrogen and oxytocin receptors in uterine epithelial cells [5, 6]. Transcriptional inactivation of the *Cox-2* gene from uterine epithelial cells, upon exposure to IFN- $\tau$  has also been suggested [7]. IFN- $\tau$  has potent antiviral [8] and antiproliferative activity [9,10] like other IFNs, but is less toxic in vitro and in vivo than IFN- $\alpha$  and IFN- $\beta$  [11, 12], moreover they are unique in that, these responses are not induced by viral infection [2], and could therefore be considered a possible alternative to IFN- $\alpha$ . IFN- $\tau$  also inhibits human and feline immunodeficiency virus replication [13, 14] without cytotoxicity, and its anti-HIV effects in human macrophages are stronger than those of IFN- $\alpha$ 2a [13]. IFN- $\tau$  induces the same antiviral pathways in macrophages as other type I IFNs but without associated toxicity [15]. This is important since the type I IFNs are potent therapeutic agents for the treatment of various diseases, moreover preventing the infection of macrophages and the production of viral particles in these cells might make it possible to control the spread of HIV to some extent.

Bovine IFN- $\tau$ , like IFN- $\alpha$ , IFN- $\beta$  and IFN- $\omega$  belong to type I IFNs, and possess many common features. IFN- $\tau$  exhibit nearly 70 and 50% homology to IFN- $\omega$  and IFN- $\alpha$  (166-residue), respectively. On the basis of secondary and tertiary structure predictions, all IFN subtypes probably fold in a roughly similar manner and have the same general overall architecture based on five  $\alpha$ -helices separated by loop regions [16]. The INF- $\tau$  family can be further sub-divided into 3 major groups. Out of different bovine isoforms of INF- $\tau$  expressed during blastocyst stage, there is a major bias for expression of IFN- $\tau$ 1c and INF- $\tau$ 3a [17]. In the present paper homology based modelling was used to predict the protein model and potential binding sites of IFN- $\tau$ 1c.

In case of humans all, type I IFNs ( $\alpha$ ,  $\beta$  and  $\omega$ ) bind to IFN receptor (IFNAR) which consists of two subunits, IFNAR-1 and IFNAR-2. IFNAR-2 binds to all types I IFNs with lower affinity and has different specificity as compared to IFNAR complex where as IFNAR-1 exhibit low intrinsic binding of IFNs but strongly affects the affinity and differential ligand specificity of the IFNAR complex [18]. Hence the molecular interaction of the IFNAR1 with bovine INF- $\tau$ 1c was carried out to predict its interaction with the human IFNAR complex.

## Methodology:

### Sequence retrieval:

The structural template protein, which is the structural homologue of the target protein i.e. INF- $\tau$ 1c from *Bos taurus*, was identified in

NCBI (www.ncbi.nlm.nih.gov/) using protein Basic Loci Alignment Search Tool (pBLAST) [19] and Protein Data Bank (PDB) [20] was set as reference data base.

### Structure Prediction and Evaluation:

Modeller 9v5 [21] was used for alignment of target protein sequence (INF- $\tau$ 1c) with its corresponding template (1B5L) with the help of align 2D module. The target sequence file and the file containing the structural coordinates of template, in the PIR (Protein Information Resource) format were generated, which are essential for identification of the common conserved sequences and to know about the active residues in both the sequences. The 3D structure was predicted based on spatial constraints.

Models generated were subjected to Swiss PDB viewer [22] for minimisation of energy, using the steepest descent conjugate gradient technique to eliminate the unfavourable contacts between protein and H $_2$ O molecules and to correct the stereochemistry of the model. Computational analysis was carried out in vacuo with GROMOS96 43B1 parameters set, without reaction field in Swiss PDB viewer. The refined model was subjected to a series of tests for validation of consistency and reliability of the model using PROCHECK\_NT [23]. Backbone structure of the protein was obtained by the assessment of  $\chi/\psi$  plots. SAVS NIH (http://nih.server.mbi.ucla.edu/SAVES/) was used to explore the non bonded interactions between different atom types.

### Molecular interaction:

INF- $\tau$ 1c predicted model was docked to human IFNAR1 using Hex 5.1 (http://www.csd.abdn.ac.uk/hex/). The atomic coordinates of human IFNAR1 was retrieved from PDB (PDB ID: 1N6V) [20]. For identification of pockets present on IFNAR1, Ligsite CSC (Conolly Surface and Degree of Conservation) software (www.scoppi.biotech.tu-dresden.de/pocket/) was used. Ligsite uses grids to detect functional sites, further, conolly surfaces and degree of conservation are used for refining. Different pockets of the receptor were obtained and arranged according to their volumes. The pocket having the maximum surface area was selected and the nearest atom to the pocket was determined using the distance tool in Swiss PDB viewer. Only this pocket was used for binding in further steps. Introduction of ligand into pocket was done using Hex 5.1. A pocket is the functionality centre of protein and has no physical existence in 3 dimensional spaces. To place a ligand in the pocket with maximum interaction, indirect approach was applied. Placing of ligand into the cavity should be such that the Vander Waal's radii of the ligand and electron cloud of the closest atom to pocket do not overlap. Parameters, such as receptor and ligand angle, twist angle, steric scan were changed to bring the ligand close to the closest atom of the pocket. Before docking all hetero molecules like ions and water molecules were removed using Hex.

**Discussion:**

The amino acid sequence of the *Bos taurus* INF- $\tau$ 1c (accession number: AAF08671)\_protein was retrieved from the SWISS PROT (<http://www.expasy.org>). The molecular weight of the protein is 19.79 KDa and its pI is 6.01 which were obtained from Sequence Manipulation Suite (<http://www.bioinformatics.org/sms2/>). The template file exhibited a sequence similarity of 80%. The target and template protein alignment was performed using ClustalW for determining the conserved regions (Figure 1).

After alignment, 20 different models were generated using Modeller 9v5, out of them best 5 were selected. The selected models were then subjected to energy minimisation using SWISS PDB viewer. The model with the lowest objective function (-10029.48), was selected and subjected to quality evaluation. Ramachandran plot of the model was obtained using PROCHECK\_NT, out of total, 94.2% of the residues were in the favourable region, 5.2% in additional allowed regions, 0.6% in generously allowed regions and 0.0% in disallowed regions. The quality factor of protein model was 87.5% which was obtained using SAVS NIH ([nihserver.mbi.ucla.edu/SAVES](http://nihserver.mbi.ucla.edu/SAVES)).

The model was then submitted to protein model data base (PM0075822) (<http://mi.caspar.it>). The visualisation of the 3D structure of protein was done with the help of PyMol software. The generated model was found to contain 5  $\alpha$  helices separated by loop regions, as reported earlier for other members of Type I IFN family. The high level of structural similarity, of different members of IFN- $\tau$  family, and among different species indicates the significance and conservation of their function.

Based on rigid-body docking using HEX 5.1, both proteins were analyzed for shape complementary and electrostatic interactions (Figure 2). Two pockets (pkt 87 & pkt 19) of IFNAR1 were obtained from Ligsite. Pkt 87 was chosen as it was having the maximum surface area and volume. Also from the Swiss PDB viewer, residue Arg 73 with atom 'C-alpha', having distance 5.06Å was found to be the closest to the pocket. It was found that keeping ligand and receptor angle at 45°, twist angle at 180° and steric scan at 15, ligand was closest to the pocket and total energy was -370.15. These docking results indicated that the complex (figure 2) could be stabilized by hydrogen bonding.

Type I IFNs induce the synthesis of antiviral factors via activation of the cellular Jak-STAT signalling pathway. There are three main mechanisms involving 2', 5'-oligoadenylate synthetase (2', 5'-OAS), RNA-dependent serine-threonine kinase R (PKR) and MxA protein. 2',5'-OAS activated by the TAR sequence on human immunodeficiency virus (HIV) RNA, activates the latent RNase L. The 2', 5'-OAS/RNase L pathway was found to be involved in inhibiting HIV. The second factor PKR, is known to impair HIV replication in chronically infected cells, and the third, MxA protein of the GTPase superfamily has been proposed to act at different levels of the virus replication cycle, as for other viruses [15]. The potential binding of the IFN- $\tau$ 1c protein with the human IFNAR1 may illicit the same response as reported earlier for ovine recombinant IFN- $\tau$ . Though production of recombinant bovine IFN- $\tau$ 1c has never been reported, other members of bovine INF have been produced but their efficacy as anti-viral or anti-HIV agents has never been explored.

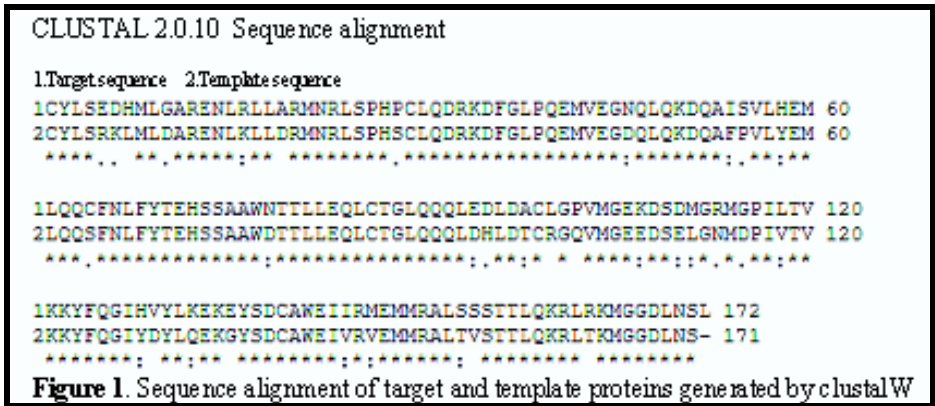


Figure 1: Sequence alignment of target and template proteins generated by clustalW

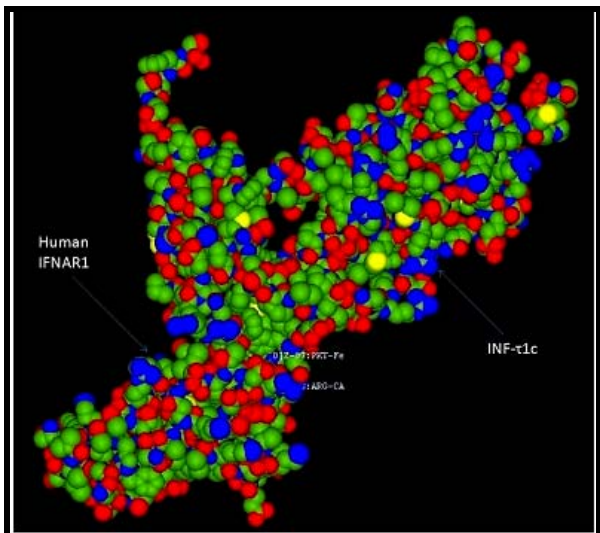


Figure 2: Molecular interaction between IFNAR1 & INF- $\tau$ 1c

**Conclusion**

Structure of INF- $\tau$ 1c was predicted using homology modelling, which is basically similar to other INFs and the crystallized murine INF- $\beta$  [24]. Proper understanding of the structure, may not only add on to the current understanding of functioning but also differences within closely related proteins of the same family. INF- $\tau$  is structurally different from other INFs ( $\alpha$ ,  $\beta$  and  $\omega$ ), biologically they constitute a distinct serological group, and also differ considerably in amino acid sequence. Also they are much less toxic than other INFs. This suggests that INF- $\tau$  may present themselves in a distinct manner, to the receptors on the uterine endometrium and other potentially functional sites, as compared to other INFs. A comparison between the structure and amino acid residues in the potential binding site may provide a clue to these differential properties. Here we have shown that bovine INF- $\tau$ 1c interact with the human IFNAR1 and therefore may bind to the human IFNAR, thus activating its downstream signalling molecules. Ability of ovine INF- $\tau$  has been previously shown to exhibit resistance to HIV-1 infection in human macrophages [15]. Thus it will be exciting to explore the antiviral activity of bovine INF- $\tau$ 1c.

**References:**

[1] RM Roberts *et al.*, *Prog Nucleic Acids Res Mol Biol.* **56**: 287 (1997) [PMID: 9187057]  
 [2] RM Roberts *et al.*, *Endocr Rev.* **13**: 432 (1992) [PMID: 1385108]  
 [3] FW Bazer *et al.*, *Am J Reprod Immunol.* **37**: 412 (1997) [PMID: 9228295]  
 [4] WW Thatcher *et al.*, *Theriogenology* **47**: 131 (1997)  
 [5] TE Spencer *et al.*, *Biol Reprod.* **53**: 732 (1995) [PMID: 7578700]  
 [6] RS Robinson *et al.*, *J Endocrinol.* **160**: 21 (1999) [PMID: 9854173]

[7] M Binelli *et al.*, *Biol Reprod.* **63**: 417 (2000) [PMID: 10906045]  
 [8] CH Pontzer *et al.*, *Biochem Biophys Res Commun.* **152**: 801 (1988) [PMID: 3365252]  
 [9] CH Pontzer *et al.*, *Cancer Res.* **51**: 5304 (1991) [PMID: 1913653]  
 [10] B Skopets *et al.*, *Vet Immunol Immunopathol.* **34**: 81 (1992) [PMID: 1441230]  
 [11] JM Soos *et al.*, *J. Immunol.* (1995) **155**: 2747. [PMID: 7544384]  
 [12] PS Subramaniam *et al.*, *Proc Natl Acad Sci USA.* **92**: 12270 (1995) [PMID: 8618883]  
 [13] N Dereuddre-Bosquet *et al.*, *J Acquir Immune Defic Syndr Hum Retrovirol.* **11**: 241 (1996) [PMID: 8603260]  
 [14] CH Pontzer *et al.*, *J Immunol.* **158**: 4351 (1997) [PMID: 9126998]  
 [15] C Rogez *et al.*, *J Virol.* **77**: 12914 (2003) [PMID:14610214]  
 [16] J Li, RM Roberts, *J Biol Chem.* **269**: 24826 (1994) [PMID: 7929162]  
 [17] AD Ealy *et al.*, *Endocrinology* **142**: 2906 (2001) [PMID: 11416010]  
 [18] EC Cutrone, JA Langer, *J Biol Chem.* **276**: 17140 (2001) [PMID: 11278538]  
 [19] SF Altschul *et al.*, *J Mol Biol.* **215**: 403 (1990) [PMID: 2231712]  
 [20] H Berman *et al.*, *Nat Struct Biol.* **10**: 980 (2003) [PMID: 14634627]  
 [21] W Kaplan, TG Littlejohn, *Brief Bioinform.* **2**: 195 (2001) [PMID: 11465736]  
 [22] J Dundas *et al.*, *Nucl. Acids Res.* **34**:W116 (2006) [PMID: 16844972]  
 [23] P Stothard *Biotechniques* **28**: 1102 (2000) [PMID: 10868275]  
 [24] Y Mitsui *et al.*, *Pharmacol Ther.* **58**: 93 (1993) [PMID: 8415875]

**Edited by P. Kanguane**

**Citation: Jamwal *et al.*, Bioinformatics 4(4): 155-157 (2009)**

**License statement:** This is an open-access article, which permits unrestricted use, distribution, and reproduction in any medium, for non-commercial purposes, provided the original author and source are credited.