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Homology Modeling of Buffalo (*Bubalus bubalis*) Interferon-Tau Protein

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ABSTRACT

Interferon-tau (IFNt) contributes towards maternal recognition of pregnancy in ruminants (like, cattle, buffalo, goat, giraffe). IFNt has been extensively studied in most of the ruminants except for buffalo *Bubalus bubalis*). The present study has been undertaken to predict the secondary structure of Interferon-tau in buffalo. The available amino acid sequence of bubaline IFNt (sequence database of SwissProt) was subjected to protein-BLAST to find similar sequences with high scores and low e-values. The ovine IFNt sequence (PDB code: 1B5L) was selected for further computational analysis of the bubaline IFNt sequence to predict the secondary and tertiary structure. The secondary structure of the modeled bubaline IFNt was predicted using STRIDE. The 3D structure was generated using academic version of MODELER9v6. The Probability Density Functions (PDF) was used to restrain Cα-Cα distances, main chain N-O distances as well as main-chain and side-chain dihedral angles. The energy minimization and van der waal contacts were taken care of using ACCELRYS DS Modeling 2.0. The residue profiles of the obtained three-dimensional models were checked by VERIFY3D. The energetic architecture and the correctness of the generated model revealed that the predicted secondary model was correct and acceptable. The predicted structure of the bubaline IFNt constructed through homology modeling from ovine IFNt, can be used for functional characterization of bubaline IFNt.

Keywords: Interferon-Tau, Buffalo, Homology Modeling, Bioinformatics

1. INTRODUCTION

Interferons (IFN), the glycoproteins belonging to the larger class of cytokines, are released in response to the invading microbial pathogens or tumor cells to exercise anti-luteolytic, antiviral and anti-proliferative effects (Cooke *et al*., 2009). Interferon-tau (IFNt) constitutes a novel type-I of interferon, which is not virus-inducible, unlike IFN-alpha and IFN-beta. The successful establishment of pregnancy is a result of the protection of the conceptus by the maternal recognition of the fetus, mediated via interferon-tau. It also displays high antiviral and anti-proliferative activities across ruminants with a prominent lack of cytotoxicity. It inhibits the endometrial oxytocin receptors in order to mute the pulsatile release of prostaglandin-F2-alpha that induces luteolysis and also increases protein synthesis in uterine glands to promote implantation of embryo. The effect of IFNt on

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the *in vitro* development of bovine embryos showed a growth promoting effect on the preimplantation blastocyst. Interferon-tau (IFNt) is constitutively secreted in high concentrations by the trophoectoderm into the uterine lumen on days 12-15 in sheep, days 14-17 in cows and goats. IFNt is transcribed from multiple autosomal genes (Walker *et al*., 2009). At least 12 distinct IFNt cDNA have been identified from bovine concepti, despite the apparent singular responsibility of the cytokine in pregnancy maintenance (Rasmussen *et al*., 2005). Isoforms and distinct clusters of IFNt that have evolved in goats have also been identified. Besides, Leukemia Inhibitory Factor (LIF) and Colony Stimulating Factors (CSF) have direct bearing on the successful implantation and completing a full term pregnancy.

Cytokines direct the events of early pregnancy and play an important role in the maternal recognition of pregnancy but the exact mechanism of action is not known till date. It is a well-established fact that the structure of a protein determines its function. The elucidation of the secondary structure will help in understanding the role of IFNt in early recognition of pregnancy and implantation of the blastocysts. In addition to its role in pregnancy, successful attempts have been made to utilize the anti-tumor activity of IFNt to formulate a therapeutic agent (Bazer *et al*., 1999). Due its wide area of applications, it is essential that the secondary structure be known to establish the functionality of the protein with its structural integrity. Attempts have been made to generate homology models of bovine and ovine IFNt based on the refined crystal structure of murine IFN-beta (MuIFN-beta) through computer modeling. The IFNt structure, like that of MuIFN-beta, is based on five long alpha helices (A-E), one short helix in the middle of the loop connecting helices C and D and a long loop between helices A and B. Bovine-IFNt differs from MuIFN-beta (Senda *et al*., 1995). However, no such reports are available on homology modeling of the buffalo IFNt. The results that we have obtained may help in further elucidating the multivariate role of interferon-tau and help understand its role in maintenance of pregnancy in the bovine embryo. The present study is an attempt to predict the secondary and three dimensional tertiary structure of Interferon-tau in bubaline so that it can help to understand the interactions during implantation of blastocysts.

2. MATERIALS AND METHODS

2.1. Template Search and Sequence Alignment

The amino acid sequence of buffalo (target) was retrieved from the sequence database of SwissProt (http://www.uniprot.org/uniprot/Q5IRM8). The three-dimensional structure of the protein was not available in Protein Data Bank, hence the present exercise of developing the 3D model of the buffalo IFNt peptide was undertaken. BLASTp (Altschul *et al*., 1990) search was performed against Brookhaven Protein Data Bank (PDB) (Bermen *et al*., 2000) with the default parameters to find suitable templates for homology modeling. Based on the maximum identity with high score and lower e-value crystal with the structure of buffalo IFNt, the Ovine interferon (PDB code: 1B5L) (Radhakrishnan *et al*., 1999) was selected as template. The sequence identity and similarity between the target and template are 73% and 83%, respectively. The buffalo protein sequence was submitted to Genesilico protein fold-recognition metaserver. Fold-recognition server Fugue and 3D PSSM reported 1B5L as the best template with highly significant score. The sequence alignment of buffalo and 1B5L was carried out using the CLUSTAL W (Thompson *et al*., 1994) program (http://www.ebi.ac.uk/clustalw).

2.2. 3D Structure Generation

The academic version of MODELER9v6 (http//:www.salilab.org/modeler) (Sali and Blundell, 1993), was used for 3D structure generation based on the information obtained from sequence alignment. The probable density function (pdf) values, estimated by MODELLER 9v6, signify the spatial restraint that are used in restraining the model structure are derived from correlations between structural features in a database of families of homologous proteins aligned on the basis of their 3D structures. These functions are used to restrain Cα-Cα distances, main chain N-O distances, main-chain and side-chain dihedral angles. The 3D model of a protein is obtained by optimization of the molecular pdf such that the model violates the input restraints as little as possible. The molecular pdf is derived as a combination of pdfs restraining individual spatial features of the whole molecule. The optimization procedure is a variable target function method that applies the conjugate gradients algorithm to positions of all non-hydrogen atoms. Out of 20 models generated by MODELLER, the models having the best G-score of PROCHECK (Laskowski *et al*., 1993) and the best VERIFY3D (Eisenberg *et al*., 1997) profile, was subjected to energy minimization. Using the parameters as a distance-dependent dielectric constant (ε) = 1.0 and non binding cutoff of 14Å, CHARMm (Brooks *et al*., 1993), force field and CHARM-all-atom charges, initially the steepest descent algorithm was used to remove close van der waals contacts, followed by conjugate gradient minimization until the energy showed stability in sequential repetition. All hydrogen atoms were included during the calculation. The energy minimization was started with main chain of the core and then all core side chains were subjected to the same. All calculations were performed by using ACCELRYS DS Modeling 2.0 (Accelrys Inc. San Diego, CA 92121, USA) software suite. During these steps, the quality of the initial model was improved. VERIFY3D (a structure evaluation server) were used to check the residue profiles of the obtained three-dimensional models. STRIDE (Leaman *et al*., 1992) was used in prediction of secondary structure of the modeled buffalo protein. In order to assess the stereo-chemical qualities of the three dimensional models PROCHECK analysis was performed.

3. RESULTS

3.1. Model Building

BLASTP search was performed against PDB with default parameters to find suitable templates for homology modeling. Based on the maximum identity with high score and lower e-value 1B5L was used as the template for homology modeling. Sequence alignment between buffalo protein and 1B5L was done using ClustalW program. The sequence–structure alignment used for model building has been shown in **Fig. 1**.

```
CLUSTAL 2.0.12 multiple sequence alignment


Buffalo         CDLSQKHMLDGRENLRLLGQMSRLSPHFCLQDRKDFAFPQEMVEGGQLQKDQAISVLHEM 60
1B5L            CYLSRKLMLDARENLKLLDRMNRLSPHSCLQDRKDFGLPQEMVEGDQLQKDQAFPVLYEM 60
                * **:* ***.****:**.:*.***** ********.:*******.*******:.**:**

Buffalo         LQQSFNLFHTERSSAAWDTTLLEQLRTGLHQQLEDLDACLGPVTGEKDSDLGRMGPTLAV 120
1B5L            LQQSFNLFYTEHSSAAWDTTLLEQLCTGLQQQLDHLDTCRGQVMGEEDSELGNMDPIVTV 120
                ********:**:************* ***:***:.**:* * * **:**:**.*.* ::*

Buffalo         KKYFQGIHVFLKEKEYSDCAWEIIRV-------------------------- 146
1B5L            KKYFQGIYDYLQEKGYSDCAWEIVRVEMMRALTVSTTLQKRLTKMGGDLNSP 172
                *******: :*:** ********:**
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Fig. 1. Sequence alignment of buffalo protein with the template (PDB code 1B5L) done using CLUSTALW server that was subsequently submitted to MODELLER. The conserved regions are indicated by '*'

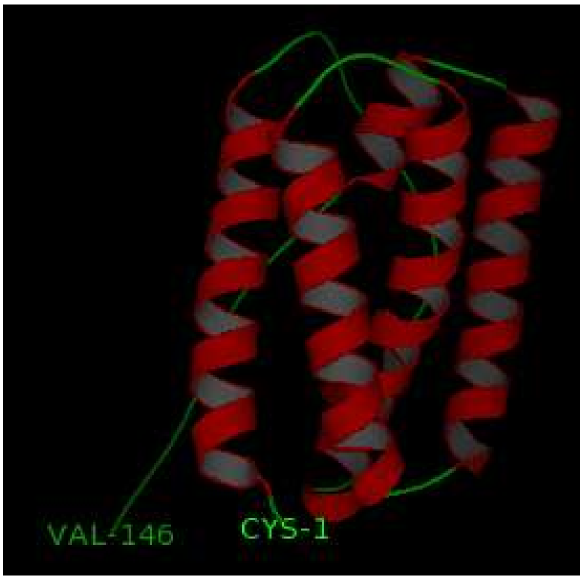
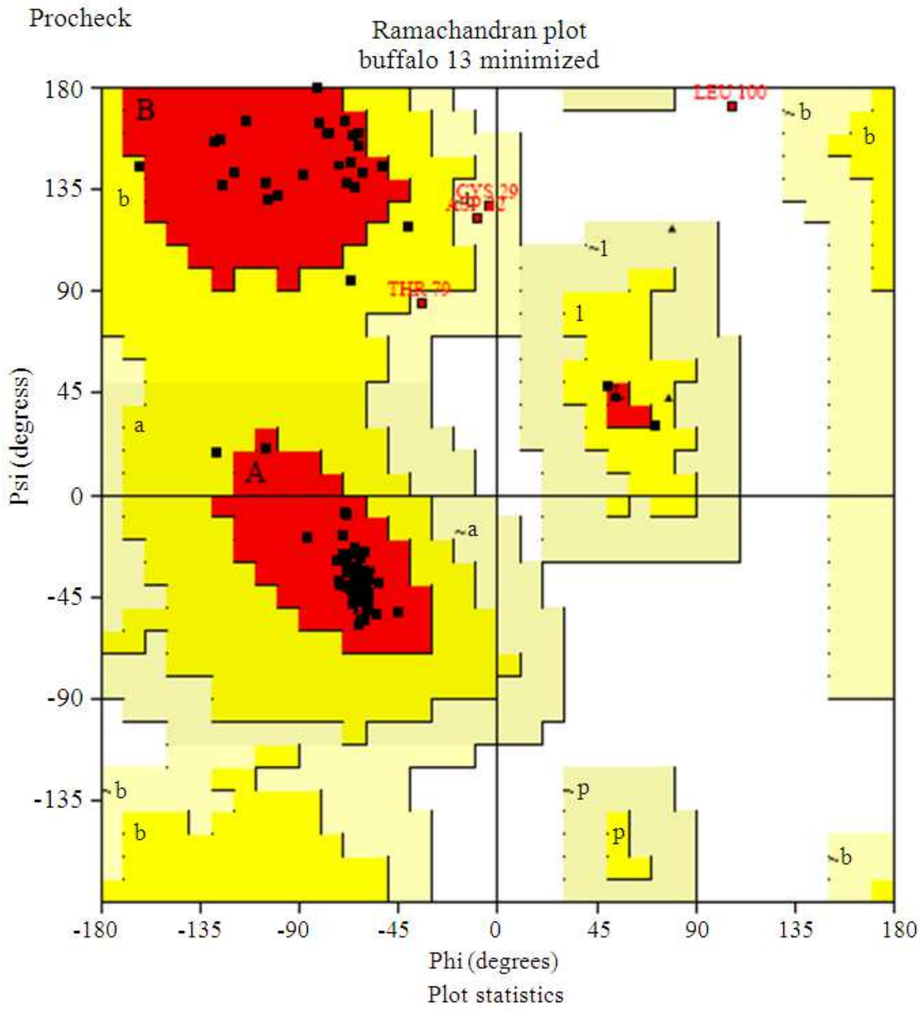


Fig. 2. Ribbon representation of modeled buffalo protein. The α-helices and β -sheets are shown as helices and ribbons, respectively. The rest are shown as loops. The figure was prepared by PyMol



Plot statistics

Residues in most favoured regions [A,B,L]	122	93.8%
Residues in additional allowed regions [a,b,l,p]	4	3.1%
Residues in generously allowed regions [~a,~b,~1,~p]	3	2.3%
Residues in disallowed regions	1	0.8%
	----	------
Number of non-glycine and non-proline residues	130	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	10	
Number of proline residues	4	

Total number of residues	146	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favored regions

Fig. 3. Ramachandran's Map of buffalo protein. The plot calculation on 3D model of buffalo protein was calculated with the PROCHECK program

Fig. 4. Superimposition of Cα trace of buffalo protein (represented in green color) and 1B5L (represented in pink color)

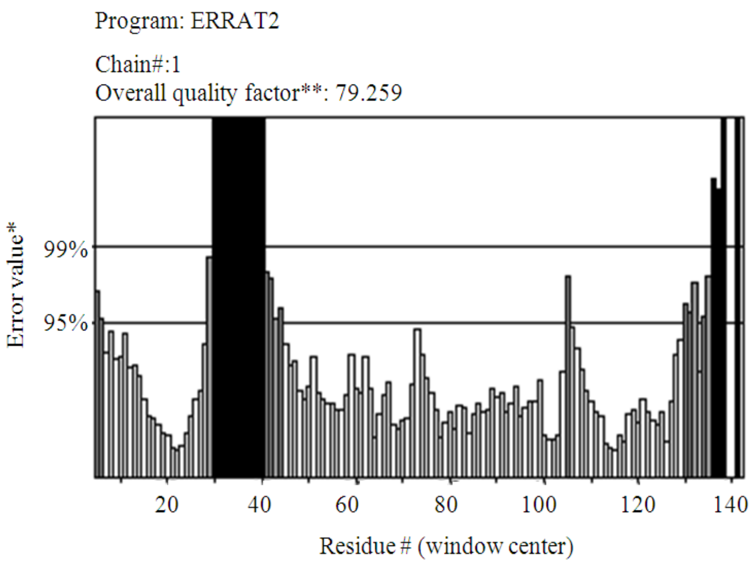


Fig. 5. The ERRAT score (79.259) as a quality factor for the predicted model. A model with ERRAT score greater than 50 is considered as good quality and acceptable model

The alignment is characterized by some insertions and deletions in the loop regions. The first 23 residues were deleted because the corresponding residues were not present in the template. Modeling was carried out from 24 to 169 residues followed by a rigorous refinement of the model by means of energy minimization using CHARMm (Brooks *et al*., 1993) force field. The final stable structure of buffalo IFNt has been shown in **Fig. 2**.

3.2. Protein Structure Validation

To validate the homology modeled buffalo protein structure, a Ramachandran plot was drawn and the structure was analyzed by PROCHECK, a well-known protein structure checking program. It was found that the phi/psi angles of 93.8% residues fell in the most favored regions, 3.1% residues lied in the additional allowed regions, 2.3% fell in generously allowed regions and 0.8% residues lied in the disallowed conformations (**Fig. 3**). The overall PROCHECK G-factor for the homology modeled structure was -0.02. This score indicates that the modeled structure is acceptable. The structural superimposition of C^{α} trace of the model over template structure 1B5L (**Fig. 4**) was resulting in a root mean square deviation (RMSD) of 1.9 Å (Z-score 6.5) using CE program (http://cl.sdsc.edu/ce.html) which indicates a valid structure of the model. For the predicted structure, the ERRAT score (also called the quality factor) was 79.259, thus supporting its good quality as score >50 is acceptable for a reasonable model (**Fig. 5**). Energetic architecture as revealed by PROSA (data not shown) score was negative (-5.87) for the modeled protein, value quite similar to that was obtained for template (-5.27), which indicates its correctness. Results of WHATCHECK also indicate about the correctness of the modeled structure. Based on these results, it was ascertained that obtained structure is of reasonably good quality.

4. DISCUSSION

The interferon-tau (IFNt) has been studied for molecular features using wet-laboratory approach in some non-primate species, like, Giraffe (Liu *et al*., 1996), Goat (Ealy *et al*., 2004), Ewes (Ott *et al*., 1997), bovine embryos (Stojkovic *et al*., 1999). Reports are available on trophoblastic interferons in other species (Ezashi *et al*., 2008; Cencic *et al*., 2003). The trophoblastic-interferons secreted in non-ruminants, during the peri-implantation period of pregnancy, are not IFNt (Mirando *et al*., 1990). In mouse conceptus, IFNt expression has not been demonstrated, however, antiviral activity in placentae has been reported. Similar findings have also been reported in equines (Bazer *et al*., 1994).

Interferon-tau has been characterized both *in silico* and experimentally in various species. Molecular characterization of the Interferon-tau gene of Mithun (*Bos frontalis*) has been done using 588 bp mithun IFNt (mitIFNt) gene and *in silico* translated 195 amino acid pre-peptide (Rajaravindra *et al*., 2006). High resolution structure of buffalo IFNt protein has not yet been determined experimentally. In a similar type of bioinformatics study, a model for the helical cytokines has been constructed by progressively improving a placement of four amphipathic helices onto training sequences was constructed using biocomputational approaches (Conklin, 2004).

Sequence alignment is a basic technique in homology modeling. We built a model following homology modeling protocol. It is used to establish a one-to-one correspondence between the amino acids of the reference protein (template) and those of the unknown protein (target) in the structurally conserved regions. The correspondence is the basis for transferring coordinates from the reference to the model protein. In general, the homology modeling method is based on the assumption that the structure of an unknown protein is similar to known structures of reference proteins. The MODELER software employs Probability Density Functions (PDFs) as the spatial restraints rather than energy (Sali and Blundell, 1993; Sali *et al*., 1993; 1995; Sali and Overington, 1994). The main chain conformation of a given residue in the model will be described by restraints that depend upon the residue type, the main chain conformation of equivalent residues in the reference proteins and the local sequence similarity.

4.1. Relationship between Secondary Structure of Bubaline IFNt and Implantation

Around 65% of all embryonic loss occurs between days 6-18 days of pregnancy. Interferon tau secreted by the trophectoderm of the conceptus (Bazer *et al*., 1994), gives the first pregnancy signal (Roberts *et al*., 1999) and prevents development of the endometrial luteolytic mechanism (Spencer and Bazer, 2002). The structural basis for ovine-IFN-tau's (onIFNt) activity profile was probed at 2.1 A resolution, by Radhakrishnan *et al*. (1999). The study revealed that the fold of ovIFNt contains five alpha-helices, similar to that of the previously determined crystal structures of human IFN-alpha2b and human and murine IFN-beta. However, comparative assessment between ovIFNt with huIFN-alpha2b, huIFN-beta and muIFN-beta reveals considerable structural differences even in regions of sequence identity. Secondary structural difference has been shown in helix A, the AB loop, helix B and the BC loop. However, until now, bubaline IFNt has not been characterized crystallographically. Our present study clearly shows that the ovine and bubaline IFNt share sequence identity and similarity between the target and template are 73 and 83%, respectively.

The ovine IFNt secondary structure, as deduced by structural prediction algorithms and circular dichroism, indicates a functionally important, primary alpha helical conformation that has been brought into close proximity. The five alpha helices of IFNt, in murine IFN-beta model, are A (residues 4-20), B (52-68), C (79-100), D (115-133) and E (138-158) (reviewed by Martal *et al*., 1998). The D helix plus the DE loop combined with part of the E helix derive from the long D helix. A three-dimensional model of IFNt was deduced from X-ray crystal structure analysis of recombinant murine IFN-beta and from circular dichroism analysis of ovine-IFNt (Jarpe *et al*., 1994). Homology study of IFNt protein can be extended for assessing cross-species similarity. Such phenomena leave ample opportunities to characterize and thereafter identify the molecular pathway contributing to the recognition of pregnancy and antimicrobial activity of IFNt in bubaline. Seemingly, it is much evident that the conformational difference among species may contribute to the ability of the female to carry out successful pregnancy up to term vis-à-vis the varying degree of tolerance against microbial infection. The present study unveils the structure of the bubaline IFNt that has been constructed by homology modeling from ovine IFNt. Further research work directed towards structure comparison among related species would help in profiling species specific differential activities of IFNt.

5. CONCLUSION

The predicted tertiary structure of the bubaline IFNt can be used for functional characterization of bubaline IFNt. The structural features of the bubaline IFNt can be comparatively studied with that of other domestic large ruminants (viz. cattle) to understand the underlying difference between these species.

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