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Cross-Species Amino Acids Sequence Comparison and Computational Docking of Human IL-1Ra and Rat IL-1Ra on Rat Receptor

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Abstract

Nowadays, experimental animals are being widely used to investigate therapeutic potentials of IL-1Ra. This article states cross-species comparison of amino acid sequences of IL-1Ra and computational docking of ligands (IL-1Ra_human and IL-1Ra_rat), on rat receptor (IL-1RI_rat). The purpose of study was to understand relationship between amino acid sequences, structural and binding similarities between both ligands. We utilized molecular modeling for structure determination, sequence alignment, structure refinement and protein docking of concerned proteins. We comparatively verified amino acid sequence of both ligands using Emboss Needle method, to express pairwise sequence. Further, these sequences were used to generate 3D structural model of ligands by Phyre2, that were verified by Ramachandran plot. Docking of ligands to structure of IL-1RI_rat was attempted by using AutoDock program. The similarity and identity of sequences and structures of IL-1Ra_human and IL-1Ra_rat were 85.4% and 73.6%, respectively. Two of three receptor residues, i.e. LYS290 and ASP259, were common in docking of both ligands. This elucidates that binding sites for both ligands are apparently similar, signifying that both ligands will probably possess same therapeutic efficacy. Similarly, functional implication of significant similarity found between two species can be of great importance in future investigations, regarding use of exogenously administered IL-1Ra.

Keywords: IL-1Ra; Sequence comparison; Emboss needle method; Ramachandran plot; Protein docking

Introduction

IL-1Ra, a naturally occurring anti-inflammatory antagonist of IL-1 cytokine family, has recently been investigated for its therapeutic effects against the treatment of autoimmune diseases and syndromes, such as rheumatoid arthritis, inflammatory bowl diseases, and diabetes mellitus [1-5]. Various studies have also verified the broad spectrum anti-inflammatory therapeutic potentials of IL-1Ra [6-8]. IL-1Ra is an endogenous protein, however under certain conditions, its production inside the body is suppressed, disturbing the balance between IL-1Ra and its IL-1 family [2-4,9], and as a result, inflammation occurs in corresponding tissues. The recombinant human IL-1Ra, before being administered to human, is generally investigated for its therapeutic outcomes on experimental animals, such as rodents against specific inflammatory diseases. Nevertheless, it is essential to cross check the sequencing of endogenous IL-1Ra of experimental animals, and the administered IL-1Ra as difference among the amino acid sequence of the two proteins, probably indicates a practically impossible target of achieving the desired therapeutic outcomes with the administered drug. Similarly, it is also crucial to confirm the amino acid sequences of desired protein in cross-species, along with the investigation of therapeutic potentials of the administered target drug. This can be achieved by utilizing the far and wide growing extents of bioinformatics tools [10]. Since last decade, the scope of bioinformatics tools is being expanded in biological and medical sciences. Through various computational tools, it has become possible to analyze protein sequences and expression, perform gene annotation, along with molecular modeling and docking [11-14].

The purpose of our present research was to compare the amino acid sequences of IL-1Ra_human with that of IL-1Ra_rat, using various bioinformatics tools and find out the percentage similarity, and/or identity between these two proteins from two different species, and binding similarities between both ligands. We also superimposed the 3D structures of IL-1Ra_human with IL-1Ra_rat and found that the amino acid sequences of IL-1Ra-human were quite similar and identical with that of amino acid sequences of IL-1Ra_rat. We also visualized the binding efficiency of both ligands on IL-1RI_rat and found that two of the three receptor residues, i.e. LYS290 and ASP259, were common in the docking of both ligands.

Materials and Methods

Comparative modeling of protein sequences

The FASTA amino acid sequences of IL-1Ra_human and IL-1Ra_rat were retrieved from Uniprot (http://www.uniprot.org/), and their Pairwise sequence alignment was determined using Emboss Needle method (http://www.ebi.ac.uk/Tools/psa/emboss_needle/). Emboss needle created optimal global alignment of the amino acid sequences of IL-1Ra_human and IL-1Ra_rat using ClustalW2 [15]. Following parameters were used to get suitable alignment results: Matrix: BLOSUM62, GAP OPEN: 10, GAP EXTENDED: 0.5, OUTPUT FORMAT: pair, END GAP PENALTY: false, OPEN GAP OPEN: 10 and END GAP EXTEND: 0.5.

For comparative analysis of IL-1Ra in two species, we also performed matching of sequence similarities using TBLASTN analysis,

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and compared using WebAct online source [16] and alignment Graph was visualized by Artemis Comparison (ACT) Tool [17].

3D protein structural modeling

The sequence of two individual proteins from human and rat was entered in turn to generate the structural model of proteins using 3D-PSSM [18], by mapping the sequences of templates with aligned residues of IL-1Ra. Phyre2 has two modes for modeling a protein, i.e. normal and intensive. We used intensive mode as we wanted to perform Multi-template modeling and *ab initio* (http://www.sbg.bio.ic.ac.uk/phyre2/html/page.cgi?id=index). The rat receptor sequence of interleukin-1 receptor type I (IL-1RI) was taken from uniprot, and was submitted to I-tassar server for protein modeling [19].

Verification of 3D structural protein model

The Phyre2 server generated two protein structural models in PDB format. Each PDB file generated in turn was submitted to Ramachandran plot server to generate Ramachandran plot, to verify 3D structural model of IL-1Ra_hmand and IL-1Ra_rat (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Protein structure refinement

The model generated by Phyre2 server for Rat was further refined by using Chiron protein minimization server (http://chiron.dokhlab.org). The correction and modification done by Chiron protein server on rat structure was checked again using ramachandran plot.

Molecular visualization of protein structural similarity

Once model verification was confirmed with Ramachandran plot, the analysis and visualization was done in PyMOL. Both files were opened in PyMOL software (DeLano, W.L. The PyMOL Molecular Graphics System, 2002, http://www.pymol.org). Human protein was red in color and rat protein was green. We used ribbon structure, as they make it easily to be visualized when are superimposed. Lastly, we opt to find the structural similarity by measuring Root-Mean-Square Deviation (RMSD), when both proteins were superimposed.

Protein docking

Pre-processing of the target protein was done by loading receptor protein into MGL tools [20], to refine the structure. It was checked to remove any monomers in the PDB files, and the required one was selected. Then, the polar hydrogen atoms were added to the receptor and finally, glisters charges were applied to apply partial charges and convert the file into PDBQT format.

Ligands were also initially visualized and processed through MGL Tools [20]. The numbers of torsion angles were limited to 32, as there was restriction by the AutoDock 4.2. AutoGrid4.2 was performed by using grid matrix of 120×120×12-matrix, using MGL tools by covering extracellular part of the receptor, according to the information from the Uniprot server.

AutoDock 4.2 was performed by using genetic algorithms, and setting the number of runs to 100 for each ligand simulation to achieve accurate results. The results generated by AutoDock 4.2 were visualized by MGL tools, and clustering was used to select docking position on the basis of number of conformation, and binding energy was also noted.

The docking sites identified by the MGL tools were further analyzed in PYMOL, using The PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC. (http://www.pymol.org/citing). Pymol was used to identify the number of bonds, and their bond length formed in docking position selected earlier in MGL tool.

Results

Pairwise sequence alignment of IL-1Ra_human and IL-1Ra_rat

To identify the most suitable structural template, amino acid sequences of concerned proteins were generated using Uniprot (http://www.uniprot.org/). We used Global alignment for the pairwise sequence comparison, as our aim was to create end to end alignment of the two sequences (Figure 1). The FASTA sequence of amino acids of IL-1Ra_human and IL-1Ra_rat, taken from Uniprot database server, were further analyzed. In case of both human and rat, we focused on sequence of the isoform 1 of IL-1Ra because the purpose of our project was restricted to isoform 1 only. To compare, the amino acid sequence of IL-1Ra of both species was aligned using Emboss Needle global alignment as shown in figure 2. The result showed that the human IL-1Ra isoform 1 shares 85.4% (152/178) similarity and 73.6% (131/178) identity with rat IL-1Ra isoform 1 (Figure 2). However, we noticed only a slight gap percentage in sequence alignment table 1.

On the base of TBLASTN analysis of two sequences, IL-1Ra_human andIL-1Ra_rat showed same similarities and same pattern of alignment, except the differences of few bases, which was also obtained from BLAST analysis (Figure 3). It indicates that both proteins did not have differences in the sequence, except few bases.

Validation of protein structure models

The models generated by Phyre 2 server were further verified, initially using Ramachandran plot. The percentage of residues in favored region in human and rat were found to be 94.3% and 78.4%, respectively. The outlier regions in IL-1Ra_human were 1.7%, whereas

```
#=====================================

IL-1RA_RAT      1 MEICRGPYSHLISLLLILLFRSESAGHPAGKRPCKMQAFRIWDTNQKTFY      50
                  ||||||..||||:||| .||.||:...|:|::..|||||||||.||||||
IL-1RA_HUMAN    1 MEICRGLRSHLITLLL-FLFHSETICRPSGRKSSKMQAFRIWDVNQKTFY      49

IL-1RA_RAT     51 LRNNQLIAGYLQGPNTKLEEKIDMVPIDFRNVFLGIHGGKLCLSCVKSGD     100
                  ||||||:|||||||||..|||||||:|||:...:||||||||:|||||||||
IL-1RA_HUMAN   50 LRNNQLVAGYLQGPNVNLEEKIDVVPIEPHALFLGIHGGKMCLSCVKSGD      99

IL-1RA_RAT    101 DTKLQLEEVNITDLNKNKEEDKRFTFIRSETGPTTSFESLACPGWFLCTT     150
                  :|:||||.||||||::|:::|||||.||||::||||||||.||||||||.
IL-1RA_HUMAN  100 ETRLQLEAVNITDLSENRKQDKRFAFIRSDSGPTTSFESAACPGWFLCTA     149

IL-1RA_RAT    151 LEADHPVSLTNTPKEPCTVTKFYFQEDQ     178
                  :|||.|||||||.|.|...||||||||||:
IL-1RA_HUMAN  150 MEADQPVSLTNMPDEGVMVTKFYFQEDE     177

#=====================================
```

Figure 1: Pairwise sequence alignment of IL-1Ra_human and IL-1Ra_rat. |: amino acid are identical in all sequences, :: conserves substitutions, .: semi-conserved substitution. Alignments were calculated using ClustalW2.

```
#=====================================
# Aligned sequences: 2
# 1: IL-1RA_RAT
# 2: IL-1RA_HUMAN
# Matrix: EBLOSUM62
# Gap penalty: 10.0
# Extend penalty: 0.5
#
# Length: 178
# Identity:      131/178 (73.6%)
# Similarity:    152/178 (85.4%)
# Gaps:            1/178 (0.6%)
# Score: 705.0
#=====================================
```

Figure 2: Comparison of amino acid sequences of IL-1Ra in two species (human and rat).

in IL-1Ra_rat, they were 11.9%, that were significantly high, as compared to that of IL-1Ra_human (Table 1).

Computer modeling of protein structure

The structures of the protein were modeled by Phyre2 protein modeling server, by using sequence of amino acids of IL-1Ra_human and IL-1Ra_rat (Figure 4). Phyre2 is a successor of 3D-PSSM and Phyre [18]. It is one of the best servers for protein modeling using amino acid sequence. The server first identify template, according to the given sequence. It uses these templates to predict the secondary structure of the given sequence. This also takes in account, disorder predication and transmembrane.

Protein structure refinement

Chiron is a protein minimization server, which performs rapid energy minimization of protein molecules using discrete molecular dynamics, with an all-atom representation for each residue in the protein [21]. The model generated by phyre2 server for Rat had 11.9% of outliers (Table 1). Therefore, rat structure modeled was further refined by using Chiron protein minimization server (http://chiron.dokhlab.org). The correction and modification done by Chiron protein server on rat structure was checked again using ramachandran plot. As shown in table 2, it reduced the outlier from 11.9% to 5.1%, and increased the number of residues in favored region from 78.4% to 85.2%. The details of this are shown in ramachandran plot (Figure 5), and superimposition of model with and without protein minimization server (Figure 6).

Molecular visualization of protein structural similarity

The protein models generated by Phyre2 were further verified

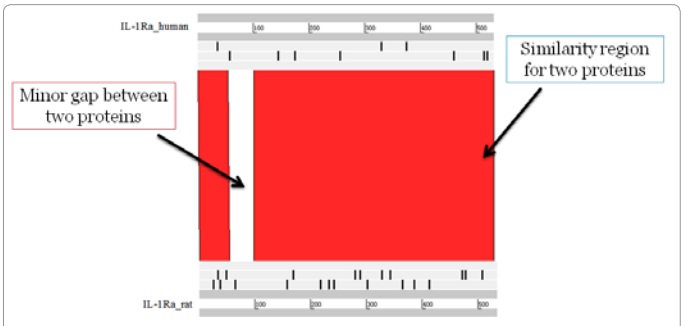

Figure 3: Comparison of sequences similarities IL-1Ra_human with IL-1Ra_rat by TBLASTN analysis using WebAct, and visualized with Artemis Comparison (ACT) Tool.

IL-1Ra_human	
Number of residues in favored region	165 (94.3%)
Number of residues in allowed region	7 (4.0%)
Number of residues in outlier region	3 (1.7%)
IL-1Ra_rat	
Number of residues in favored region	138 (78.4%)
Number of residues in allowed region	17 (9.7%)
Number of residues in outlier region	21 (11.9%)

Table 1: Ramachandran Plot calculations for 3D model of IL-1Ra in human and rat. All values were computed with PROCHECK program.

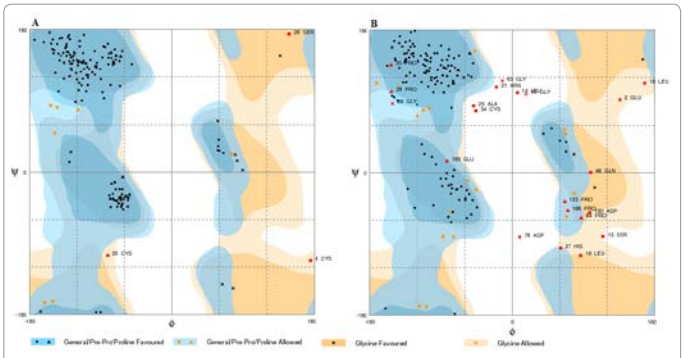

Figure 4: Ramachandran map of IL-1Ra_human (A) and IL-1Ra_rat (B) on 3D model of IL-1Ra was calculated by PROCHECK program.

Modified values of IL-1Ra for rat	
Number of residues in favored region	150 (85.2%)
Number of residues in allowed region	17 (9.7%)
Number of residues in outlier region	9 (5.1%)

Table 2: Protein structure refinement. The residual values of IL-1Ra in rat were refined by using Chiron protein minimization server (http://chiron.dokhlab.org).

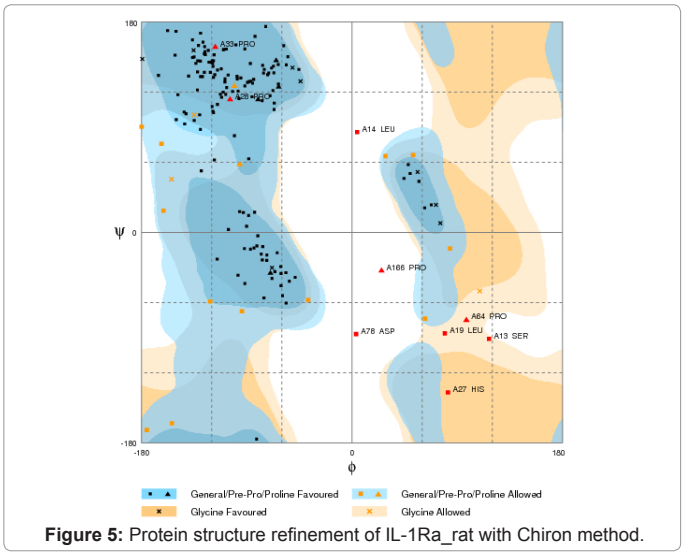

Figure 5: Protein structure refinement of IL-1Ra_rat with Chiron method.

by minimizing the energy, using amber plug-in in PyMOL. Further validity of these models was done using PROCHECK [22]. These validated models of proteins were than superimposed to locate the structural similarity among them, using PyMOL. As we have noticed gap percentage in sequence alignment in tables 1 and 2, therefore, human 35-176 Cα chain was superimposed to rat 36-177 Cα chain, as shown in figure 7. The RMSD was found to be very low, about 0.276 (142 to 142 atoms).

Protein docking

Docking of ligand (IL-1Ra_human and IL-1Ra_rat) to the structure of receptor (IL-1RI_rat) was attempted by using AutoDock program. The results were analyzed after visualizing the protein by MGL, that generated the grids for binding site. An exhausted search was performed using Lamarckian Genetic Algorithm (LGA), using 100 runs which enabled the docking to be most accurate. Figures 8A and 8B represents the best docking model of IL-1Ra_human and IL-1Ra_rat proteins

bound to IL-1RI_rat. Moreover, the docking positions between the ligand residues and receptor residues, along with their respective bond lengths, were analyzed to achieve the best docking positions as shown in table 3. Two of the three receptor residues namely, LYS290 and ASP259, were observed to be common for the docking of both ligands. The binding energy of IL-1Ra_human was 175 kcal/J, whereas for IL-1Ra_rat, it was 208 kcal/J.

Discussion

Use of computer modeling to predict 3D protein structure is an alternate tool for the investigation of structural and functional relationship of desired protein in cross-species. In our present work, our attempt was to investigate the relationship of structural and functional similarity of IL-1Ra between human and rat. Therefore, in order to gain insight into our objectives, we applied various bioinformatics tools to compare the amino acid sequencing of IL-1Ra between two species, and develop a structural and functional similarity of designated protein between human and rat. Bioinformatics tools may help to compare the amino acid, and/or DNA sequencing of two and/or multiple available proteins. We used one of the best targets for sequence alignment of protein using automatic server, which was followed by 3D modeling, based on best fitted sequence alignment. Finally, the predicted 3D structural model was compared with 3D structure of other species.

Emboss Needle is a pair wise sequence alignment tool, used to identify similar region between two amino acid sequences of target proteins in different species that indicate their structural, functional and evolutionary relationship. The relatively high level of sequence identity between human IL-1Ra isoform 1 and rat IL-1Ra isoform 1 as interpreted in table 2 signifies that both sequences possibly will share similar structure and function. From these results, it is also evident that if this human protein is incorporated in rat or *vice versa*, it will perform the same function.

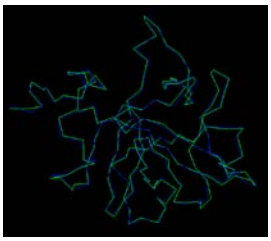

Figure 6: Superimposition ofIL-1Ra_rat before (represented in blue color) and after (represented in green color) refinement.

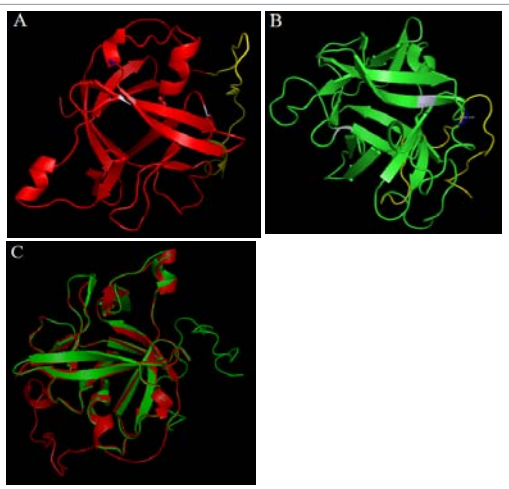

Figure 7: Computer generated 3D modeling of IL-1Ra_human (A) and IL-1Ra_rat (B). Ribbon representation of 3D model shows the whole molecule of IL-1Ra. Superimposition of Cα-chains of IL-1Ra_human (represented in red in color) and IL-1Ra_rat (represented in green in color).

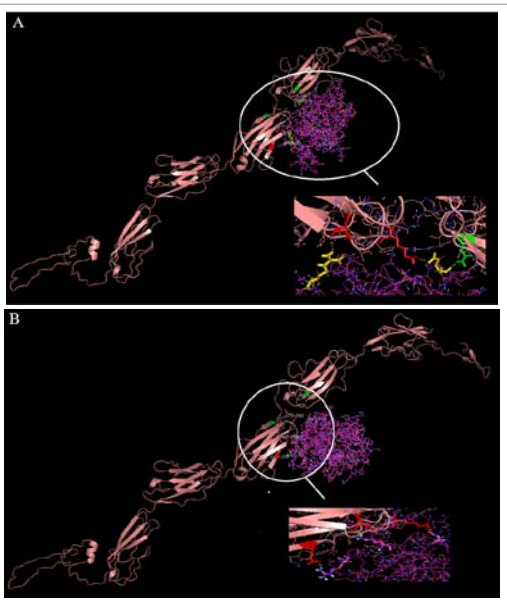

Figure 8: Protein docking of IL-1Ra_human (A) and IL-1Ra_rat (B) with IL-1RI_rat.

IL-1Ra_human		
Rat Receptor Residues	Ligand residues	Bond Length
LYS290	GLU175	2.7
ASN196	GLU175	2.0
ASP259	ARG26	2.7
IL-1Ra_rat		
Rat Receptor Residues	Ligand residues	Bond Length
LYS290	ASN110	2.0
GLU279	ARG32	1.9
ASP259	LYS35	2.2

Table 3: Docking positions of ligand residues on receptor residue with respective bond lengths.

Ramachandran plot is a tool which computes models of small polypeptides to systematically vary phi (ϕ) and psi (Ψ), with the aim of finding the stable confirmations. For every confirmation, the structure was examined closely between atoms. So phi and psi angle which causes the sphere to collide were identified and corresponded to sterically disallowed conformations of polypeptide backbone. The rat structure model was further refined by using Chiron protein minimization server, as it showed more outlier region. This finally resulted into a refined model, with about 85.2% of favored region.

Moreover, PyMOL, which is an open source molecular visualization system created by DeLano scientific was used as a tool to visualize and analyze the concerned proteins. One of the very important functions of this tool is that it shows structural similarities between proteins, when they are superimposed. The RMSD was found to be 0.276 (142 to 142 atoms), therefore, these structures might probably be considered similar to each other regarding their functionalities. This may signify

least possibility of hyper-reactivity, when IL-1Ra is administered to cross-species (rat, and/or human).

Protein docking is one of an essential computational tool that helps predict the best intermolecular binding sites for two or more concerned molecules. The identification of bonds was done at hydrogen cut off of 3.6 and bond cut off edge of 3.2. This enabled us to identify bonds between residues of receptor, and each ligand to be more accurate as shown in figures 8A and 8B. Interestingly from the results of table 3, it has been clearly found that receptor residues, i.e. LYS290 and ASP259, were involved in the docking of both ligands. This elucidates that the binding sites for IL-1Ra_human and IL-1Ra_rat on the IL-1RI_rat are apparently similar to each other, which further signifies that both ligands will probably possess the same therapeutic efficacy. Due to less value of binding energy of IL-1Ra_human, as compared to that of IL-1Ra_rat, the binding strength of IL-1Ra_human was stronger than that of IL-1Ra_rat.

Conclusion and Future Perspectives

To conclude, a comparative pairwise amino acid sequencing of IL-1Ra_rat and IL-1Ra_human has been made, along with further comparison of their functional and structural relationship. Based on structural and sequencing similarities found between IL-1Ra_rat and IL-1Ra_human in the present work, the functional implication of the significant similarity found between the two species can be of great importance in future investigations, regarding the use of exogenously administered portentous IL-1Ra. Moreover, in the docking model of IL-1Ra_human and IL-1Ra_rat binding to IL-1RI_rat, we found that there were two common amino acids in their binding. However, further structural modification of protein may also help depict more probable functions related to this protein.

Conflict of Interest

Authors declare that they do not have any conflict of interest for this article.

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