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Homology Modelling and Molecular Docking Studies of Interleukin 10 Proteins from Different Species

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ABSTRACT

Background: Interleukin-10 is an important anti-inflammatory cytokine that plays significant roles in auto immune diseases. Interleukin-10 has been involved as an important regulator of the functions of myeloid cells lymphoid cells. The three-Dimensional structure and functions of Interleukin-10, proteins from different species are not known. In the present study, we analyzed the comparative study of 3D structure of eight different species of Interleukin-10 proteins and also molecular docking studies were performed to all the modelled structures. **Methods:** Comparative modelling was performed to all the selected eight proteins by using Modeller 9.21, a modelling tool. Initial alignment was performed by using clustal X and validated by using Procheck. Further molecular docking study was performed by using Autodock 4.2. **Results and Discussion:** Homology modeling studies of all the modelled proteins showed that all the amino acid residues present in core region and there is no amino acid residue in disallowed region. A molecular docking study was also carried out to study the stability of Interleukin-10 proteins. Docking studies were performed by using natural compounds as Interleukin-10 protein inhibitors. All the compounds exhibited good binding energies and good interactions. Binding energies ranged from -4.51 to -9.82 Kcal/mol. **Conclusion:** Homology modelling and docking study results indicate that the natural compounds are showing good interactions with all the modelled interleukin-10 proteins. To this study we get significant information for the design of novel inhibitors for the treatment of inflammation.

Key words: Interleukin-10, Homology modelling, Natural Compounds, Molecular modeler, Docking.

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INTRODUCTION

The counter inflammatory cytokine Interleukin 10 (IL-10), identified by Mosmann and associates in 1989 is a negative controller of immune reactions. Loss of IL-10 prompts inflammatory diseases.[1] IL-10 was at first depicted as a T helper 2 (32)- derived cytokine, however, it was later acknowledged that IL-10 isn't confined to certain T cell subsets yet found in practically all leukocytes.[2] An important role of IL-10 in atherosclerosis has emerged by discovering its ability to manipulate lipid metabolism in macrophages. Mouse (m)IL-10 and human (h)IL-10 genes are encoded by five exons on chromosome 1, rmouse (r)IL-10 quality is encoded by 4 exons on chromosome 13 and each come under epigenetic control.[3,4] Interleukin-10 additionally exhibits a variety of immune-stimulatory consequences for select cell types and these exercises might be limited to specific species. These include improvement of *in vitro* viability and up-regulation of MHC class II molecule expression by highly purified dense B cells obtained from the spleen of unstimulated mice.[5] Further research into IL-10 treatments has distinguished key deterrents, for example, the quick clearing of IL-10 from the damage site and its inability to cross the blood–spinal cord barrier.[6] IL-10 immunosuppressive movement is mediated by heterodimeric IL-10 receptor (IL-10R1, IL-10R2). Despite the fact that the IL-10 receptor complex is observed at different degrees in a myriad of cell types; monocytes and macrophages seem, by all accounts, to be the essential target of IL-10. Receptor ligation initiates JAK/STAT signalling, prompting huge changes in the expression profile of immunomodulatory genes,[7] which, in actuality, serve to restrain the arrival of proinflammatory mediators, decline antigen introduction and phagocytosis and associatively enhance the inhibitory, resilience and scavenger elements of these cells. IL-10, similar transforming growth factor beta (TGF-β), is also an administrative cytokine with pleiotropic functions in the immune resistance network. In any case, the primary task of TGF-β is to maintain T cell resilience to self or harmless ecological antigens by means of its direct effects on separation and homeostasis of effector and regulatory T cells. Deficiencies in the TGF-β pathway bring about hyper activation and uncontrolled development of T cells prompting a deadly multi-organ immune system issue.[8] Interestingly, IL-10 functions essentially as an input inhibitor of extreme T cell reactions to microbial antigens. Most noticeably, IL-10-deficient mice immediately display colitis, exhibiting that IL-10 has a basic job in keeping up peripheral immune resilience.[9] Furthermore, through arrival of mitigating particles like interleukin-1 receptor antagonist (IL-1RA), solvent TNF-α receptor and interleukin 27 (IL-27) or by means of physical interactions with T lymphocytes, IL-10 can in a straightforward or in a roundabout way restrain the development of 31 cells.[10]

Taking all of the above characteristics mentioned above, we can hence conclude that IL-10 plays an important role in the regulation and functioning of our immune response and tolerance and any mutation or deficiency could leave our body susceptible to a wide range of infections which may be mild and harmless or deadly and lethal.

Hence, the aim of the present study is to build a three-dimensional structure of eight different Interleukin-10 proteins from different species by using the homology modeling. In addition, this study also focuses on performing the identification of the effective inhibitory activity of

known broad spectrum anti-inflammatory compounds using molecular docking studies.

MATERIALS AND METHODS

Sequence alignment, comparative protein modeling and model validation

The protein sequences of *Interleukin 10* isolate *Rattus norvegicus (UniprotKB: P29456)*, *Oryctolagus cuniculus (UniprotKB: Q9TSJ4)*, *Gallus gallus (UniprotKB: Q6A2H4)*, *Vulpes vulpes (UniprotKB: Q25BC1)*, *Callithrix jacchus (UniprotKB: Q0Z972)*, *Meriones unguiculatus (UniprotKB: P47965)*, *Sus scrofa (UniprotKB: Q29055), Macaca mulatta (UniprotKB: P51496)* were obtained from UniprotKB[11] portal. To find related protein templates in order to build models for these primary sequences, a sequence similarity search has been carried out separately by using Protein BLAST[12] tool against solved protein structures deposited in Protein Data Bank (PDB). ClustalX and ClustalW2 is used for the correction of alignment.[13] MODELLER 9.21[14] was used to gain satisfactory models.[15] MODELLER is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints which employs position dependent gap penalties based on structural information of the template for generating alignments.[16] After manually modifying the alignment input file in MODELLER 9.21 to match the template and query sequence, 20 models were generated and minimized using the molecular dynamics and simulation procedure CHARMM (Laskowski *et al*. 1993) the last step of homology modeling, the selected model was subjected to a series of tests for its internal consistency and reliability. Backbone conformation was evaluated by the inspection of the psi/phi Ramachandran plot obtained from PROCHECK[17] analysis.

Active site prediction

The active site for docking was predicted using Tripos Sybyl6.7 SiteID[18] module after adding hydrogen atoms to the modeled proteins. The potential binding sites were predicted and identified by the SiteID module by correlating and combining key criteria such as depth, exposure, temperature factor, hydrophobicity, solvent accessible surface and hydrogen-bonding capability. While detecting the potential binding sites, the SiteID module generates necessary files required for performing Autodock4.2 docking studies.

Docking studies

Molecular docking studies were performed to elucidate the binding mode of interleukin-10 proteins and the ligands. A total of ten molecules were docked to the protein to locate the appropriate binding orientations and conformations of various inhibitors in the binding pocket using the Graphical user Interface program "Auto-Dock 4.2".[19] AutoDock 4.2 is an automatic docking tool designed to predict how small molecules bind to a receptor of 3D structures, which generates grids and calculates the dock score to evaluate the conformers. Gasteiger - Huckel united atom charges, polar hydrogen's and solvation parameters were added to the receptor for the preparation of protein in docking simulation, Gasteiger - Huckel charges assigned and then non-polar hydrogens were merged as the docking ligands were not peptides. All torsions were allowed to rotate during docking. The generation of PDBQT files for protein and ligands preparation, grid box creation was carried out with AutoDock Tools 1.5.6 (ADT).[20] AutoDock saved the prepared ligand file and the PDB file in PDBQT format. Generated AutoGrid was used for the preparation of the grid map using a grid box. The grid size was set to 60 × 60 × 60 XYZ points and grid center was designated at dimensions (x, y and z). In Autodock, both the protein and ligands are considered as rigid. The binding poses with the lowest docked energy belonging to the top-ranked cluster was selected as the final model for post-docking analysis.

RESULTS

A 3-Dimensional structures of different *interleukin-10* proteins were built using Modeller9.21. The protein sequences of *Interleukin 10* isolate *Rattus norvegicus (UniprotKB: P29456)*, *Oryctolagus cuniculus (UniprotKB: Q9TSJ4)*, *Gallus gallus (UniprotKB: Q6A2H4)*, *Vulpes vulpes (UniprotKB: Q25BC1)*, *Callithrix jacchus (UniprotKB: Q0Z972)*, *Meriones unguiculatus (UniprotKB: P47965)*, *Sus scrofa (UniprotKB: Q29055), Macaca mulatta (UniprotKB: P51496)* were selected from uniprot and selected template with 88% identity (PDB: 4X51 with resolution 2.05 Å). All the models showed more than 95% of amino acid residues in core region. *Out of 8 different species Rattus norvegicus and Meriones unguiculatus showed 96.9% of amino acid residues in most favored region.*

The amino acids fall under active site for docking was predicted using Tripos Sybyl 6.7 SiteID module and the active site is shown in Table 2. During the docking procedure, selected only the best fit active site pocket with respect to the ligands in order to dock them. AutoDock 4.2 was used for molecular docking studies. Results obtained from AutoDock 4.2 provided information on the binding orientation of ligand-receptor interactions. Free energies of binding (ΔGb) and dissociation constants (Ki) as calculated by AutoDock are summarized in the Tables 3 and 4. Table 3 summarizes molecular docking interactions and binding energies of Q29055_*Sus scrofa protein. This species showed highest bind-*

Table 1: Percentage of Amino acid residues falling in different regions of Ramachandran plot of proteins.

S. No	Name of the species	Core region		Allowed region		Generously allowed region		Disallowed region	
		No of residues	%	No of residues	%	No of residues	%	No of residues	%
1	*Rattus norvegicus*	157	96.9	4	2.5	1	0.6	0	0
2	*Gallus gallus*	155	95.7	6	3.7	1	0.6	0	0
3	*Vulpes vulpes*	154	95.1	6	3.7	2	1.2	0	0
4	*Oryctolagus cuniculus*	151	95.0	7	4.4	1	0.6	0	0
5	*Callithrix jacchus*	155	95.7	5	3.1	2	1.2	0	0
6	*Meriones unguiculatus*	157	96.9	4	2.5	1	0.6	0	0
7	*Sus scrofa*	153	97.5	3	1.9	1	0.6	0	0
8	*Macaca mulatta*	155	95.7	6	3.7	1	0.6	0	0

Table 2: Ligand binding sites as detected by the SiteID module of Tripos Sybyl6.7 software.

S.no	Name of the species	Active site of protein
1	*Rattus norvegicus*	Phe55, Met95, Leu109, Leu112
2	*Gallus gallus*	Leu40, Ile47, Leu92, Met106, Leu109, Leu113
3	*Vulpes vulpes*	Val52, Phe56, Ala99, Ile106, Val110, Leu113
4	*Oryctolagus cuniculus*	Gly35, Gly36, Leu37, Pro38, Leu41, Arg42, Arg45
5	*Callithrix jacchus*	Val51, Phe55, Met95, His108, Val109, Leu112
6	*Meriones unguiculatus*	Val51, Met95, Leu109, Leu112
7	*Sus scrofa*	Cys76, Leu79, Glu124, Phe125, Pro127
8	*Macaca mulatta*	Arg128, Phe129, Pro131, Gly79, Cys80, Leu83

Table 3: Binding energy and interacting amino acids against compounds in Q29055_*Sus scrofa protein.*

S.No	Ligand	Interactions	Binding Energy ΔG (Kcal/Mol)	Disassociation Constant
1	Acacetin	Cys76, Val16, Lys71	-8.40	695.13nM
2	Apigenin	Cys76, Val16, Lys71	-8.04	1.27 μM
3	Chrysin	Cys76, Val16, Lys71	-8.18	1.01 μM
4	Daidzein	Gly72, Cys76	-7.57	2.83 μM
5	Genistein	Ser19, Ile20, Gly72, Cys76	-7.55	2.92 μM
6	Hesperetin	Cys76, Val16, Lys71	-8.40	690.86 nM
7	Kaempferol	Cys76, Val16, Lys71	-8.03	1.29 μM
8	Luteolin	Cys76, Val16, Lys71	-7.61	2.66 μM
9	Morusin	Val16(2)	-9.82	63.33nM
10	Myricetin	Cys76, Val16(2), Lys71, Glu124	-5.46	98.8 μM

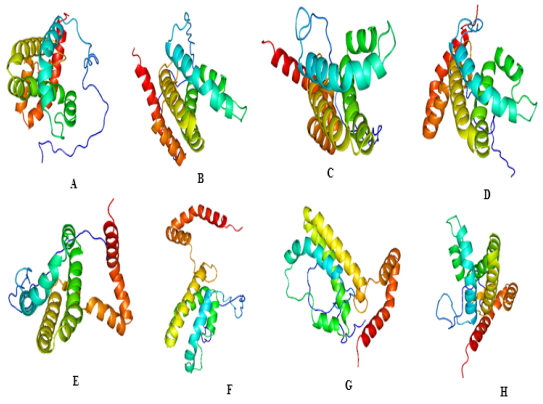

Figure 1: Homology models of Interleukin 10 proteins from different species [A]. *Rattus norvegicus (UniprotKB: P29456)*, [B]. *Meriones unguiculatus (UniprotKB: P47965)* [C]. *Macaca mulatta (UniprotKB: P51496)*, [D]. *Callithrix jacchus (UniprotKB: Q0Z972)*, [E]. *Gallus gallus (UniprotKB: Q6A2H4)*, [F]. *Oryctolagus cuniculus (UniprotKB: Q9TSJ4)*, [G]. *Vulpes vulpes (UniprotKB: Q25BC1)*, [H]. *Sus scrofa (UniprotKB: Q29055).*

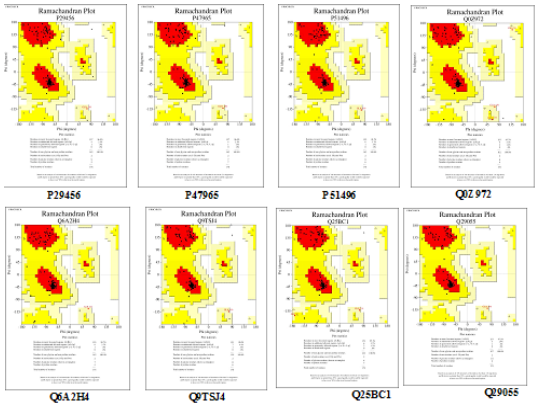

Figure 2: Ramachandran plots of modelled Interleukin 10 proteins from different species [A]. *Rattus norvegicus (UniprotKB: P29456)*, [B]. *Meriones unguiculatus (UniprotKB: P47965)* [C]. *Macaca mulatta (UniprotKB: P51496)*, [D]. *Callithrix jacchus (UniprotKB: Q0Z972)*, [E]. *Gallus gallus (UniprotKB: Q6A2H4)*, [F]. *Oryctolagus cuniculus (UniprotKB: Q9TSJ4)*, [G]. *Vulpes vulpes (UniprotKB: Q25BC1)*, [H]. *Sus scrofa (UniprotKB: Q29055).*

ing energies than other species. The modeled protein was docked with natural compounds.

DISCUSSION

After BLAST of the primary sequences of Interleukin-10 proteins with the predetermined structures deposited in PDB bank, sequences that showed the greatest similarity were considered as template sequences. Twenty models were generated using the MODELLER 9.21 program based on the sequence alignment files generated by Clustal X program. The alignment file was tweaked manually to best fit the sequences. Among the generated models for all the primary sequences, the model with least object function was selected for further evaluation for protein stereochemistry (phi and psi angles) with PROCHECK software. The final models were validated by using PROCHECK. The PROCHECK software generates a number of files which lists complete residue by residue data and the assessment of overall quality of generated structure compared to well refined structures of the same resolution. The final models were validated by using PROCHECK. The PROCHECK software generates a number of files which lists complete residue by residue data and the assessment of overall quality of generated structure compared to well refined structures of the same resolution. Figure 1 shows the predicted homology models of all the eight Interleukin-10 proteins belonging to difference species. The Ramachandran plots for all the generated models are shown in Figure 2. Table 1 shows the residues falling in different regions of Ramachandran plot. The results show that there are no amino acids falling in disallowed region of the plot and very minor percentage of amino acids in the generously allowed regions which indicate that the models are the better conformational structures. The template (PDB ID: 4X51) protein showed 94.3% of amino acid residues in most favored region, 4.9% of amino acid residues in additionally allowed region, 0.8% of amino acids in generously allowed region and 0% of amino acids in disallowed region. Loop building was performed by using SPDBV.

The docking programs place both the protein-ligand molecule in various orientations, conformational positions and the lowest energy confirmations which are energetically favorable are evaluated and analyzed for interactions. All the eight molecules that were docked showed good interactions. From the docking simulation, we observed free energy charge

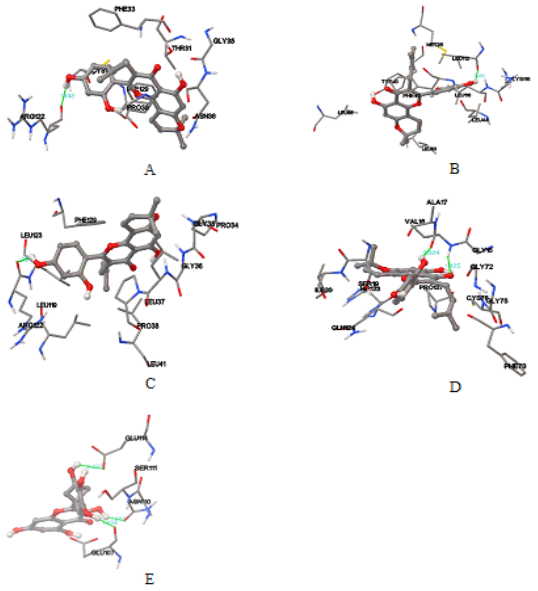

Figure 3: Interacting amino acids with highest score in different species: [A]. *Macaca mulatta (UniprotKB: P51496),* [B]. *Rattus norvegicus (UniprotKB: P29456),* [C]. *Oryctolagus cuniculus (UniprotKB: Q9TSJ4),* [D]. *Sus scrofa (UniprotKB: Q29055),* [E]. *Callithrix jacchus (UniprotKB: Q0Z972).*

of binding for the protein-ligand complex. A possible explanation may be that the radio-graphical structure of the protein from crystals differs from that of the aqueous system. Therefore, it can be concluded that the interaction of all the compounds with the corresponding targets is hydrophobic interactions in nature. Molecular docking indicated that the distance of hydrogen bonding between the ligand and the protein, respectively. Molecular interaction between Morusin with different targets, calculated the docking score. The modelled protein were used for molecular docking analysis. It was found that Morusin made hydrogen bond with Val16 (2) with binding energy of -9.82 kcal/mol a Q29055_ *Sus scrofa* Interleukin-10 protein. The compound also showed best interactions Arg122 with binding energy of -8.32 kcal/mol with P51496_*Macaca mulatta* protein. The compound Morusin showed highest binding energy with seven modelled proteins except Q0Z972_ *Callithrix jacchus species*. Total ten natural compounds were docked with all the eight modelled proteins and the results of all the docked conformations and interacting amino acids are shown in table and Figure 3.

CONCLUSION

Homology Modelling and Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. The use of informatics and complementary experimental techniques increase the chances of success in many stages of the drug discovery process. For selection of potential antiviral compounds based on its interaction, determination of the 3D structure and active site prediction for modelled interleukin-10 proteins is of the utmost importance. Therefore, the use of combinatorial approaches may result in the rapid development of better anti-inflammatory inhibitors in future. Successful docking methods search high-dimensional spaces effectively and use a scoring function that correctly ranks candidate dockings. Molecular docking studies of the above anti-inflammatory compounds showed favorable interactions in the binding site of the interleukin-10 proteins from different species. Therefore, it is concluded that these molecules are the potential candidates for anti-inflammatory drug discovery, which need to undergo further *in vivo* laboratory trials. Studies on the structure-activity inhibitions of anti-inflammatory compounds in inhibiting interleukin-10 proteins hopefully will identify targets and allow logical approach to identify chemicals that can be used as future anti-inflammatory drugs candidates.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

IL-10: Interleukin-10, **PDB:** Protein Data Bank, **SPDBV:** Swiss PDB viewer.

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