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Investigating the Binding Ligands of IL-5 Using Computational Approaches

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Abstract: Computational approaches have been widely used to study both physical and life phenomena beyond the traditional purview of computer science. In this study, web services and computer algorithms were used to study the binding ligands of Interleukin-5 (IL-5) which is one of the critical cytokines in the immune system. Ramachandran analysis with visual plot was carried out. The structural perspective of IL-5 ligand binding was studied to determine the outliers. This study contributes to the understanding of the structural binding of IL-5 ligands that may lead to a complete picture of signaling pathways in the immunological context.

Key words: Cytokine, interleukin-5, web services, bioinformatics, database, algorithm

INTRODUCTION

Structural information of macromolecules and small proteins such as cytokines and growth factors is important to provide a foundation for the understanding of various cellular signaling pathways (Wang *et al*., 2009). Cellular signaling pathways are always complex and primarily governed by the genetic regulatory mechanisms and structural interactions between ligands and receptors (Chakraborty and Das, 2010). In human immune system, the structural information of various interleukins has been examined extensively using both molecular and computational approaches (Chakraborty and Das, 2010; Ponomarenko *et al*., 2011). Interleukin is a cytokine produced by leukocytes for the regulation of immune responses that plays a pivotal role in the healthy and pathological context. The typical interleukin receptor signaling is either found in the form of ligand-mediated dimerization or reorganization of the preformed dimers (Spangler *et al*., 2015; Syed *et al*., 1998). The proper structural form of interleukin topological interaction has been reported to be implicated in homeostasis (Boyman and Sprent, 2014; Pipkin *et al*., 2010) tumorigenesis (Villarreal and Weiner, 2014), inflammatory suppression (Rutz and Ouyang, 2011), cytotoxicity (Pipkin *et al*., 2010), viral infection (Pellegrini *et al*., 2011) and hypersensitivity (Umetsu and DeKruyff, 2006; Lloyd, 2010).

Interleukin-5 (IL-5) has been known as a cytokine that implicates in immuno-regulatory pathways in general and allergen-induced eosinophilia in particular (Larose *et al*., 2014). Overexpression of IL-5 is correlated to the increase in eosinophil count, severe of which will lead to eosinophilia (Larose *et al*., 2014). Eosinophilia occurs when the amount of eosinophil in blood or tissues is higher than the normal range, usually in response to the stimuli such as IL-5, IL-3, eotaxin and Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) (Rosenberg *et al*., 2013; Rothenberg and Hogan, 2006). Eosinophilia is knowingly caused by various diseases such as allergies, skin disorder, tumors, parasitic infection, gastrointestinal disorders and eosinophilic myositis (Rosenberg *et al*., 2013; Krahn *et al*., 2006).

IL-5 is a homodimeric glycoprotein that is produced by mast cells upon stimulation with allergens (Takatsu *et al*., 1997). It can also be produced by type 2 helper T cells (Th2) after stimulation with antigens 18. IL-5 binds to its receptor (IL-5R) that consists of α chain and β chain (Moon *et al*., 2001). The α chain is putatively involved in B-cell proliferation and differentiation (Moon *et al*., 2001) whereas the β chain is indispensable in signal transduction (Kouro and Takatsu, 2009). The expression of IL-5Rα in the bone marrow cells is reported to be a determining factor in eosinophil lineage commitment (Kouro and Takatsu, 2009). Upon stimulation by IL-5 it follows a torrent of tyrosine phosphorylation of various proteins in the target cells such as Src-Homology 2 (SH2)/SH3-containing proteins, Lyn tyrosine kinase, mitogen-activated protein kinases, Janus Kinase 1/2 (JAK1/2) and signal transducer and activator of transcription 1/3/5 (STAT1/3/5) (Kouro and Takatsu, 2009; Hogan *et al*., 2008). The IL-5-induced activation of JAK2 and STAT5 pathways is important for signal transduction in eosinophils (Kagami *et al*., 2000) which has a role in the pathogenesis. In B-2 cells IL-5 plays a

similar role in triggering various transcription factors and kinases including STAT5, JAK2, Btk and Phosphoinositide 3 kinase (PI3 kinase) (Takatsu, 1998). The activated signaling cascading pathways lead to the transcription of the target genes in the nucleus which may further induce inflammatory responses by recruiting immune cells and chemokines to the target tissues.

The structural study of IL-5 can be done using wet laboratory experiment or computational approach, depending on the goal of the study. As with other proteins, the conformation of IL-5 always dictates its function. The insight of the geometry of a protein domain is always gleaned from the three dimensional studies (Vogel *et al*., 2004; Xie *et al*., 2012). Computational approaches have been recognized as an indispensable tool in shedding biological insight by complementing the experiment which is too costly or implausible to carry out. Structural information of proteins has been constructed by using various computational means (Trinh *et al*., 2012; (Amemiya *et al*., 2012). Protein motion classification has been adopted to investigate if the domain motions are coupled with ligand binding (Amemiya *et al*., 2012). To study conformational transition pre-and post-binding protein structure pairs were identified (Chang *et al*., 2012). Protein block structural alphabet had been used for the protein structure comparison (Gelly *et al*., 2011). Algorithms and mathematical techniques are used extensively to achieve various goals of structural and functional studies of protein (Du *et al*., 2009; Malekpour *et al*., 2009). In this study, algorithms and web services were used to investigate the binding ligands of IL-5.

MATERIALS AND METHODS

The database of The National Center for Biotechnology Information (NCBI) has been queried for the FASTA information about IL-5. The web server at the Protein Data Bank (Japan) had been used to identify the PDB ID and the relevant structural and molecular details (Kinjo *et al*., 2012). The sequence specific NMR resonance details for IL-5 were obtained.

In this study, Ramachandran plot (Ramachandran *et al*., 1963; Lovell *et al*., 2003) was used to obtain the details of backbone dihedral angles ψ against φ of amino acid residues. The data on dipeptide and tripeptide structure and polypeptide chains were verified to ensure the configurations were adhered to the allowed ranges of the angular parameters (Ramachandran *et al*., 1963). Frenet frames were computed by treating the protein backbone as a collection of discrete curves which is a set of sequential points that can be thought of as atoms (Quine *et al*., 2004). Covalent bonds are represented by the atom-joining line segments. The backbone of the protein can be represented as –C'—N—C_α—C', running from N- to C-terminus (Quine *et al*., 2004). Let:

$$S_j = | P_{j+1} - P_j | \quad (1)$$

we define a unit tangent vector at point P_j where j = 0, ..., n-1 as such:

$$t_j = \frac{P_{j+1} - P_j}{S_j} \quad (2)$$

The points of the curve were computed from the translation of the sequences $\{t_j\}$ and $\{s_j\}$ by:

$$P_k - P_0 = \sum_{j=0}^{k-1} s_j t_j \quad (3)$$

ProBiS web service (Konc and Janezic, 2012) was used to detect the structurally similar binding sites in IL-5. ProBiS database is deposited with a hefty pre-calculated structural similarity profile of proteins from which de novo similarity calculation was performed using ProBiS algorithm (Konc and Janezic, 2012). ProBiS algorithm aligns the folded proteins if they have similar binding sites (Konc and Janezic, 2012). The structural significance of local structure alignments in proteins was calculated as such (Konc *et al*., 2012):

$$S = \log\left(\frac{n_{vert} \times \log(1+1/e_{value})}{rmsd}\right) \quad (4)$$

Where:

S = The alignment score
n_{vert} = The number of aligned vertices
e_{value} = The alignment expectation value derived from Karlin-Altschul equation
rmsd = The root mean square deviation between pairs of superimposed vertices (Konc *et al*., 2012)

S is then standardized into Z-score to imply the statistical and structural significance of protein alignment (Konc *et al*., 2012).

Queried protein and other proteins were compared, using the maximum clique algorithm to identify the largest similar subgraph of the compared protein graphs (Konc and Janezic, 2007, 2012). The maximum clique

algorithm was used to choose a vertex with a maximum color among the vertices from a set of protein. The algorithm is shown Algorithm 1 (Tomita and Seki, 2003).

Algorithm 1; The maximum clique algorithm:

Procedure MaxClique (R,C)
1. while R ≠ 0 do
2. choose a vertex p with a maximum color C(p) from set: R
3. R: = R\{p}
4. if |Q|+C(p)>|Q_{max}|then
5. Q: = Q ∪ {p;}
6. if R∩Γ (p)≠ 0 then
7. obtain a vertex-coloting C' of G (R∩Γ(p))
8. MaxClique (R∩Γ (p), C')
9. else if |Q|>|Q_{max}| then Q_{max}; = Q
10. else return
11. end while

RESULTS AND DISCUSSION

More than twenty ligands have been identified using ProBiS web service and algorithm. Due to the space limitation, only one ligand which is of clinical significance is presented here for Ramachandran plot. Figure 1 shown the 3D structural view of human respiratory syncytial virus fusion protein core (PDB ID: 1G2C), a ligand for IL-5. This protein has three chains (A, C, E). Figure 2 shows the corresponding subunits of 1G2C.

Ramachandran analysis has been carried out for 1G2C. Figure 3 illustrate a general case while the plot for isoleucine and valine is shown in Fig. 4. Plots for pre-proline, glycine, trans-proline and cis-proline are not shown.

From the visual plots depicted in Fig. 3 and 4 and that of pre-proline, glycine, trans-proline and cis-proline, Ramachandran analysis shows that 96.7% of all residues of the fusion protein (1G2C) were in the favored regions. In addition, 99.2% of all residues were falling in the allowed regions. There were 8 outliers identified for 1G2C, as listed in Table 1.

The outliers are the bond angle values deviating by more than a few standard deviations. Given that 99.2% contours of the residues were in the allowed regions, the structural deviation of 1G2C is not problematic. From the validation study there were no outliers for bond length, chirality and planarity. Only two bond angle outliers were identified (residue 480 Pro and residue 481 LEU) of which one of them (LEU) is matched with the outlier finding from Ramachandran analysis (Table 1). This has pointed to the bond angle distortion of LEU. From the validation study, the observed bond angle of LEU (residue 481) is 96.40 degree whereas the ideal angle is 111 degree. Due to the fact that only a handful of outliers were identified, 1G2C is a structurally potential ligand for IL-5.

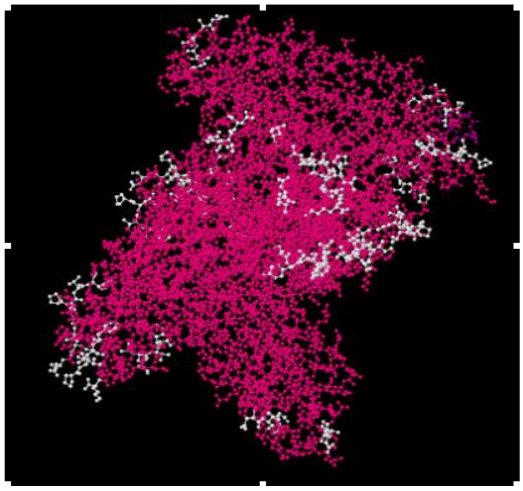

Fig. 1: Ball and stick model of 1G2C

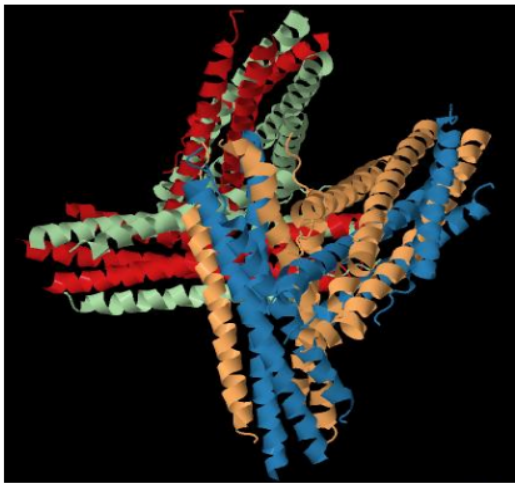

Fig. 2: Subunits of 1G2C

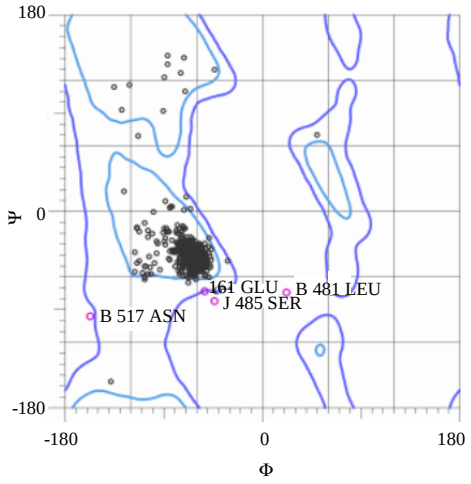

Fig. 3: A general view of Ramachandran plot for 1G2C

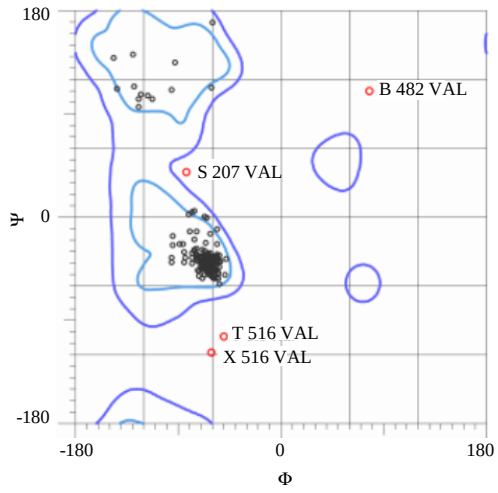

Fig. 4: Ramachandran plot for isoleucine and valine of 1G2C

Table 1: Outliers of 1G2C, an IL-5 ligand

Residues	Amino acids	(φ, ψ)
161	GLU	(-53.3, -73.1)
207	VAL	(-83.6, 40.8)
481	LEU	(22.9,-74.5)
482	VAL	(78.5,111.6)
485	SER	(-44.1, -82.7)
516	VAL	(-50.2, -104.3)
516	VAL	(-61.9, -118.2)
517	ASN	(-158.2, -96.6)

CONCLUSION

Structural study of IL-5 ligand binding is significant for the understanding of the downstream signaling pathways and the relevant transcriptional networks. A precise structural insight of IL-5 may lead to a comprehensive picture of how this cytokine interacts with other substrates in the pathological context. Subsequently it fosters a better pharmaceutical and therapy strategy. In this study, it is shown that human respiratory syncytial virus fusion protein core (PDB ID: 1G2C) is one of the ligands of IL-5. A Ramachandran analysis has performed and few outliers have been identified. In future, a network of IL-5 ligands will be probed to gain a complete picture of the role of IL-5 in the immunological context.

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