International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 2, 2011

Research Article

THEORETICAL UNDERSTANDING OF C–H….π INTERACTIONS AND THEIR DISTRIBUTION IN IMMUNOGLOBULIN PROTEINS- INSILCO GEOMETRICAL APPROACH

IFTIKHAR ASLAM TAYUBI*[1], RAO SETHUMADHAVAN#[1]

#Senior Professor, [1]Bioinformatics Division, School of Biosciences and Technology, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India; *[1]Bioinformatics Division, School of Biosciences and Technology, Vellore Institute of Technology, Vellore 632014, Tamil Nadu, India, Email: rsethumadhavan@vit.ac.in

Received: 07 Dec 2010, Revised and Accepted: 09 Jan 2011

ABSTRACT

The contribution of weakly interactions involving of C–H….π interactions, in this study we have analyzed the influence of C–H…π interactions on the structural stability of immunoglobulin proteins. In the 33 data set, a total of 128 C–H.π interactions. The most prominent representatives are the interactions between aromatic C–H donor groups and aromatic π acceptors. 97% percent of the interactions between side chain to side chain and remaining 3% percent of the C–H….π interactions were observed between side chain to side-chain five-membered aromatic ring. Long-range C–H….π interactions are the predominant type of interactions in Immunoglobulin proteins data set. The secondary structure preference, solvent accessibility and stabilization centers of C–H….π interacting residues were estimated. Moreover, the study shows that 46% of the donor residues and 52% of the acceptor residues are highly conserved. It is concluded that the C -H….π interaction can, indeed, be categorized as a true stabilizing force in Immunoglobulin proteins

.**Keywords**: C–H….π interactions; Secondary structure; Interactions range; Solvent accessibility; Conservation score.

INTRODUCTION

The importance of C–H….π interactions has been stressed by several investigators for their role in enhancement of the stability of thermophilic proteins[1, 2], folding of polypeptides[3,4] and the stability of membrane proteins [5,6] 'Immunoglobulin proteins is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. Immunoglobulin (Ig) was reported to be the most populous family of proteins in the human genome[7]. The molecules are categorized as members of this Super family based on shared structural features with "immunoglobulin proteins" they all possess a domain known as an immunoglobulin domain or fold.

The vertebrate immune system has developed into a highly sophisticated system that gives a rapid, measured and localized response to a vast variety of pathogens. They are commonly associated with roles in the immune system[8].In this study we consider the features of these proteins, how they interact and their structural stability. Protein structural stability is characterized by many factors. Non-covalent interactions such as hydrogen bonding, salt bridges, hydrophobicity, etc, have been extensively studied for their structural contributions[9], [10] In addition, C–H….π interaction is becoming more and more emphasized in modern chemistry, especially in the fields of biochemistry and biophysical chemistry and it is considered as an important interaction in the stability of three dimensional protein structures. In proteins, C–H….π interactions occur between the C atom of main- or side-chain amino acid residue and the aromatic side chains of phenylalanine (F), tyrosine (Y), tryptophan (W) and histidine (H). The exothermic

dissolution of benzene and similar compounds (π –electron system/proton acceptor) in chloroform (C–H group/proton acceptor) was perhaps the origin of an interaction, now known as C–H….π interactions[11].In 1957, Reeves and Schneider showed by NMR that this interaction was a type of H bond[12].Since then, C–H...π interactions have been described in a vast number of small molecule systems involving in interactions. In 1998, Nishio et al. published excellent treatise of these observations[13].In this way, C–H….π interactions are gradually gaining a lot of importance. They are a kind of weak hydrogen bonds. The cases in which C–H….π interactions have been described in proteins include the formation of complexes of proteins with special ligands or cofactors such as the heme group, pyridoxal-5-phosphate[14], nucleotides[15,16], carbohydrates[17] and bound peptides[18], or special geometric circumstances, for instance between neighboring side-chains around a cis peptide bond[19]. The importance of this interaction has also been recognized in the design of serine protease inhibitors[20, 21]. There are also recent reviews[22, 23] and monographs[13] where the role of C–H….π interactions in the structure of chemical and biological macromolecules is described.

These interactions also play an important role in the interaction between protein structures[15]. This kind of study is useful to understand the relation between occurrences of C–H….π interactions within the protein to the structural stability. Hence, in this work, an effort has been made to collect the information concerning C–H….π interactions in the 'Immunoglobulin proteins' in addition, we analyze the characteristic features of residues that are forming C–H….π interactions with the aid of several properties such as, secondary structure involvement, solvent accessibility, interaction range, stabilization centers and conservation score. We emphasize that all the proteins in our data set showed a C–H….π interactions, our analysis reveal that C–H….π is very significant in the sense that C–H….π interactions in 'Immunoglobulin proteins' do play a major role in structural stability of these proteins. It is noteworthy to mention here that the percentage of C–H….π interactions is higher than the percentage of cation–π interactions in the same set of proteins studied [24]. The frequency of occurrence and extent of conservation presented unequivocally shows that the C-H…π interactions cannot and must not be neglected. Hence, we emphasize that C–H….π interactions and the knowledge of this report will further help to understand structural stability of Immunoglobulin proteins and for using Immunoglobulin proteins as peptide based drug.

MATERIALS AND METHODS

Dataset

The protocol we have used for our current study in "Immunoglobulin proteins" in terms of PDB IDs of the proteins available in literature[25]. This set has been obtained with the following conditions: (i) the three dimensional structures of these proteins have been solved with ≤2.5 A ° resolution, (ii) the similarity search using PSI-BLAST yielded the e-value of less than 0.001 and (iii) the sequence identity is less than 80%. The complexes, whose proteins were homologous but recognized different nucleotide sequences, were included in the PDB IDs.

The PDB tags of the proteins are: 1igm, 1wz1, 1cfv, 1a4j, 1a4k, 1fl3, 1nbv, 1ktr, 1mpa, 1cbv, 1ap2, 1cgs, 1vpo, 1sbs, 1bbd, 32c2, 1afv,

1a6w, 1l7t, 1um5, 1jpt, 1ken, 1mci, 1a8j, 2mpa, 1e6o, 1mfb, 1mck, 1mcp, 1l6x, 1k6q, 1h3u, and 2gj7.

METHODOLOGY

C–H….π Interactions

C–H….π interactions are calculated using the program available for this purpose called HBAT[26]. The C–H….π interactions considered here are between all possible donor C–H groups in protein structures (Cα–H, Cali–H, and Caro–H) and between all side-chain π systems (the aromatic rings of Phe, Tyr, Trp, and His). The positions and geometry of donor and acceptor atom with their default parameters are shown in (Fig. 1). The donor group is represented as C–H and the acceptor is the π system. The distances are usually measured from the centroid (M), i.e., center of the π ring. P1 and P2 are distances from C and H; respectively to M. P3 is the angle between vectors C–H and H–M while P4 is the angle between the CM and MN. Here, N is a normal to the center of the π ring. The geometry is adapted from earlier work of Babu[27].

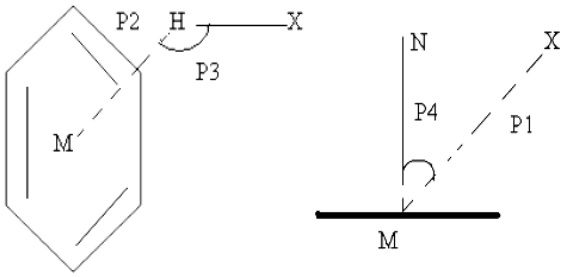

Fig. 1: Parameters for C-H….π interactions: P1≤5.00Å; P2≤4.50Å; P3≥120°.

The C–H….π interaction types are represented by a two-letter code in which the first letter indicates the donor atom and the second the acceptor: M, S and S5 represent the main-chain atom, side-chain atom and side-chain atom in the five membered aromatic rings, respectively. We classified the C–H….π interactions into four types of C–H….π interactions, namely, main-chain to side-chain C–H….π interactions (MS–C–H….π), main-chain to side-chain five-membered aromatic ring C–H….π interactions (MS5–C–H….π), side-chain to side-chain C–H….π interactions (SS–C–H….π), and side-chain to side-chain five-membered aromatic ring C–H….π interactions (SS5–C–H….π)26.

Solvent accessibility of interaction forming residue

We have estimated the solvent accessibility of all residues that are involved in C–H….π interactions in of 'Immunoglobulin protein' at various range of solvent accessibility, such as: 0–20% (buried), 20–50% (partially buried), and >50% (surface exposed), by using the program ASA-View28. Solvent accessibility was divided into three classes buried, partially buried, and exposed, indicating respectively the least, moderate and high accessibility of the amino acid residues to the solvent29, 30.

C–H….π interaction forming residues in different secondary structures

We have calculated the occurrence of interaction forming residues and its preferences in different secondary structures of Immunoglobulin proteins we found that Immunoglobulin proteins the C–H….π interaction forming interaction forming Phe, Tyr, Trp, His all prefers to be in β strand. Some contribution is also observed in Coil, βTurn, α Helix same percentage of cation–π interactions in the same set of proteins studied [24].

Sequential distance

The C–H….π interacting residues coming within a sphere of 8Å was computed as describedearlier[31–33]. For a given residue, the comparison of the surrounding residue is analyzed in terms of the location at the sequence level. The residues that are within a distance of two residues are considered to contribute to short-range interactions, whereas those within a distance of ±3 or ±4 residues contribute to medium range, and those with more than four residues away contribute to long-range interactions[34]. This classification enables us to evaluate the contribution of short-, medium-, and long-range contacts in the formation of C–H….π interactions.

Stabilization centers

Stabilization centers are clusters of residues that are involved in medium or long-range interactions[35]. Residues can be considered part of stabilization centers if they are involved in medium or long-range interactions and if two supporting residues can be selected from both of their flanking tetra peptides, which together with the central residues form at least seven out of the nine possible contacts. The stabilization centers for the C–H...π interacting amino acid residues were computed using the SCide server[36] for computing the stabilization centers.

Conservation score

We computed the conservation score of C–H….π interacting amino acid residues in each protein using the Consurf server[37]. This server computes the conservation based on the comparison of the sequence of a PDB chain with the proteins deposited in Swiss-Prot[38] and finds the ones that are homologous to the PDB sequence. The number of PSI-BLAST iterations and the E value cutoff used in all similarity searches were 1 and 0.001, respectively. All the sequences that are evolutionarily related with each one of the proteins in the data set were used in the subsequent multiple alignments. Based on these protein sequence alignments, the residues are classified into nine categories from highly variable to highly conserved. Residues with a score of 1 are considered highly variable, and residues with a score of 9 are considered highly conserved.

RESULTS AND DISCUSSION

C–H….π interactions

There are four types of C–H….π interactions, namely, main-chain to side-chain interactions (MS–C–H….π), main-chain to side-chain five-membered aromatic ring C–H….π interactions (MS5–C–H….π), side-chain to side-chain C–H….π interactions (SS C–H….π), and side-chain to side-chain five membered aromatic ring C–H….π interactions (SS5–C–H….π)[26]. As a representative picture, the SS–C– H….π interactions in Immunoglobulin proteins 1BBDare shown in (Fig.2).We found that 97% of the interactions were SS–C–H….π, and 3% interactions were SS5–C– H….π. This is shown in (Fig.3). There is no MS–C–H….π and MS5–C–H….π interactions found in the 'Immunoglobulin proteins' data set studied in this work.

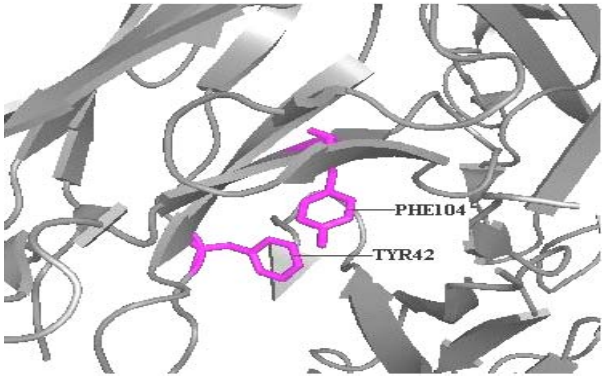

Fig. 2: PyMol view of Phe104-Tyr42 interacting pairs in 1BBD

Even though C–H….π interaction has been reported with His acting as an acceptor, the frequency of occurrence of such bonds is low owing to the unsuitability of imidazole ring in this role when charged. This might be the reason for the very less percentage of SS5–C–H….π interactions.

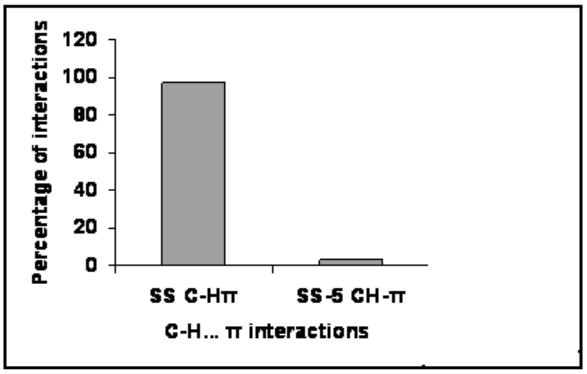

Fig. 3: C-H....π interactions types in Immunoglobulin Proteins.

Contribution amino acid residues in C–H....π interactions

The distance between the each C-H....π interactions within the 'Immunoglobulin proteins'. The structure was assessed and the distance with respect to its position of C-atom and H-atom were calculated. The C–H....π interactions forming residues distance with respect to its position of C atom is shown in (Fig.4).

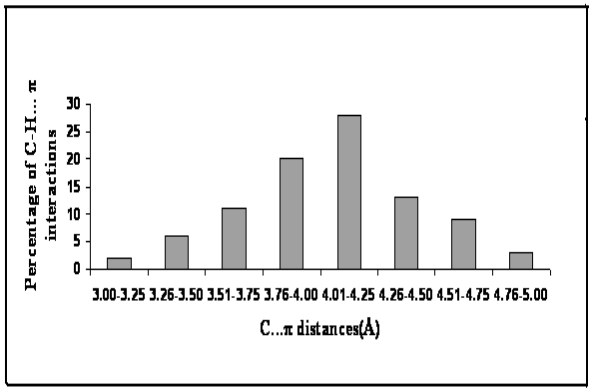

Fig. 4:.C....π interacting distances in membrane proteins

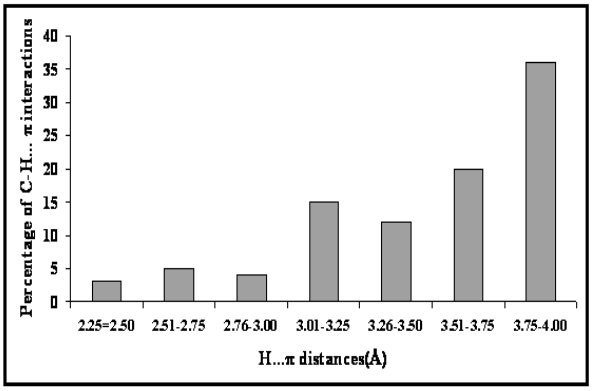

Fig. 5:.H....π interacting distances in Immunoglobulin proteins.

It was found that majority of the 28% of the interactions were found between the residue distances in the range of 4.01 to 4.25Å. The C–H....π interactions forming residues distance with respect to its position of H atom were shown in (Fig.5). Of the total 128 interactions, the majority of the 36% of the interactions were found between the residue distances in the range of 3.76 to 4.00Å.

Secondary structure preferences

The propensity of the amino acid residues to favor a particular conformation has been well documented. Such conformational preference is dependent not only on the amino acid alone but also on the local amino acid sequence. We analyzed the secondary structure preference of each amino acid, which participated in all the different types of C–H....π interactions, namely, SS–C–H....π and SS5–C–H....π interactions. The secondary structure preference of each of the amino acids involved in all the above said types of C–H....π interactions were obtained using DSSP[39] server, and the results are depicted in (Table. No.1). It is interesting to note that majority of the residues involved in C–H....π interactions such as Phe, Trp Tyr and His were preferred to be in β Strand**.**

Table 1: Frequency of occurrence of C–H....π interaction forming residue in different secondary structures

Amino Acids	Coil	βStrand	βTurn	αHelix
Phe	5.6	63.75	14.8	25.9
Tyr	12.40	44.00	37.6	6.0
Trp	6.0	64.4	26.2	6.9
His	9.0	42.5	28.7	17.8

The above characteristic features might be due to presence of all β protein structures and the presence of several -strands in Immunoglobulin proteins Thus, we can deduce that residues in β Strand have a high tendency to form the C–H....π interactions in 'Immunoglobulin proteins'.

Solvent accessibility

The relation between the amino acid residues in C–H....π interactions and solvent accessibility is depicted in (Table No.2). The solvent accessibility of amino acid residues has been categorized as buried, partially buried and exposed[28]. We found that all the aromatic residues such as Phe, Tyr, Trp and His residues were preferred to be in buried regions. This observation is quite reasonable in the sense that the aromatic residues are in principle non polar residues and tend to be buried. According to Weiss et al.[40], C–H....π interactions involving aromatic residues either as donor or as acceptor group are found mostly in the interior of the protein and tend to be buried in nature. These might be one of the reasons for their nature of solvent accessibility.

Table 2: Solvent accessibility of C-H...π interactions forming acceptor residues in immunoglobulin proteins

Amino acids	Buried	Partially buried	Exposed
Phe	65.7	29.6	4.7
Tyr	59.3	37.4	5.3
Trp	64.2	27.6	8.2
His	53.5	39.9	6.6

Sequential Separation

The contribution of C–H....π interactions in 'Immunoglobulin protein' could define either the local or the global stability of the proteins. Therefore, there is a need to evaluate the contribution of inter-residual C–H....π interactions. The residues that are within a distance of two residues are considered to contribute to short-range interactions, whereas those within a distance of ±3 or ±4 residues contribute to medium range, and those with more than four residues away contribute to long-range interactions[31-33]. As shown in (Table. No. 3)**.**

Table 3: C-H....π interaction forming residue, interacting distance, and dseq in immunoglobulin proteins

PDB CODE	Donor Residue	Donor Atoms	Acceptor Residue	disHM	disXM	D_{seq}	Total no of Interactions
1IGM	PHE 98	CE2	TR5 36	3.553	4.612	62	6
	TRP 111	CH2	TR5 36	2.786	3.582	75	

	TYR 32	CD1	TYR 91	3.945	4.847	59	
	TRP 111	CD1	TYR 95	3.480	4.196	16	
	PHE 108	CD1	HIS 99	3.591	3.900	9	
	PHE 108	CD2	TR5 111	3.186	4.167	3	
1WZ1	TRP 107	CE3	HIS 39	3.850	4.716	68	4
	TYR 54	CD2	TR5107	3.092	3.675	53	
	TYR 105	CD2	TR5 107	3.650	4.540	2	
	PHE 60	CE2	TYR 110	3.613	4.364	50	
1CFV	PHE 60	CE2	TYR 108	3.735	4.417	48	1
1A4J	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
1A4K	TYR 32	CE2	HIS 40	3.419	4.501	10	1
1FL3	TYR 178	CE2	PHE 149	3.958	4.931	29	5
	TYR 39	CE1	TYR 41	3.889	4.844	2	
	TYR 92	CD2	TYR 41	3.737	4.168	51	
	PHE 144	CD2	HIS 203	3.839	4.655	59	
	TYR 39	CE1	TYR 41	3.889	4.844	2	
1NBV	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
1KTR	TYR 40	CE2	HIS 33	3.320	4.307	7	3
	TYR 39	CD2	TYR 29	3.942	4.647	10	
	TYR 40	CE2	HIS 50	3.950	3.892	10	
1MPA	TYR 37	CE2	HIS 31	3.194	4.271	6	3
	PHE 101	CD1	TYR 54	3.417	4.348	47	
	TYR 27	CD1	TYR 100	2.775	3.847	53	
1CBV	PHE 103	CE2	TYR 41	3.681	4.615	62	8
	TRP 111	CH2	PHE 103	3.523	4.161	8	
	TYR 191	CD1	TYR 197	3.864	4.323	5	
	TYR 197	CD2	PHE 214	3.223	4.095	17	
	PHE 108	CE1	TR5 47	3.995	4.695	61	
	TRP 111	CE3	TYR 97	3.577	4.594	14	
	PHE 108	CE2	TR5 107	3.820	3.832	1	
	TYR 153	CD2	HIS 207	3.552	4.337	54	
1AP2	TRP 56	CD1	TYR 38	3.908	4.248	18	5
	TYR 105	CE2	TYR 55	3.413	4.417	50	
	TYR 55	CE2	TR5 56	3.939	4.835	1	
	TYR 105	CD2	TR5 56	3.693	4.037	49	
	TYR 55	CD2	TR5 56	3.623	4.555	1	
1CGS	TYR 37	CD2	HIS 39	3.491	4.522	2	13
	PHE 169	CE2	PHE 140	3.521	4.231	29	
	PHE 214	CE2	TYR 197	2.650	3.522	17	
	PHE 144	CD2	HIS 203	2.695	3.434	59	
	TYR 191	CE1	PHE 214	3.176	4.202	23	
	PHE 70	CE1	TR5 36	3.565	3.638	34	
	TYR 101	CD2	TR5 47	3.485	3.395	54	
	TRP 36	CD1	PHE 70	3.985	3.903	34	
	TRP 106	CE3	TYR 95	3.749	4.347	11	
	TYR 41	CE2	TR5 106	3.677	4.519	55	
	TYR 178	CD2	TYR 148	3.786	4.594	30	
	TYR 178	CE2	PHE 149	3.475	4.151	24	
	TYR 148	CD2	HIS 202	3.099	3.860	54	
1VPO	TYR 99	CD2	TYR 54	3.840	4.619	45	6
	TYR 41	CE2	TYR 94	3.682	4.655	53	
	PHE 27	CD2	TYR 32	3.811	4.579	5	
	PHE 60	CD1	TYR 99	3.643	4.044	39	
	TYR 180	CE2	PHE 151	3.723	4.584	29	
	TYR 150	CD2	HIS 204	3.600	4.380	54	
1SBS.	TYR 97	CD2	TYR 108	3.903	4.861	11	8
	TYR 42	CE2	TR5 113	3.957	4.552	71	
	TYR 38	CE1	TYR 31	3.882	3.716	7	
	PHE 104	CE2	TYR 42	3.489	4.506	62	
	TRP 56	CD1	TYR 55	3.988	4.364	1	
	TYR 97	CE2	TR5 56	3.248	4.117	41	
	TYR 55	CD2	TR5 56	3.948	4.772	1	
	PHE 68	CE1	TYR 92	3.841	4.770	24	
1BBD.	PHE 104	CE2	TYR 42	3.283	4.129	62	10
	PHE 141	CE2	PHE 124	3.827	4.524	17	
	PHE 171	CE1	PHE 141	3.449	4.211	30	
	TYR 179	CE1	TYR 146	3.411	4.394	33	
	TYR 192	CD1	TYR 198	3.942	4.212	6	
	TYR 198	CD1	PHE 215	3.843	4.543	17	
	TYR 100	CD2	TR5 47	3.506	4.435	53	
	TRP 108	CE3	TYR 95	3.763	4.535	13	
	TYR 180	CE2	PHE 151	3.714	4.680	39	
	PHE 145	CD2	HIS 204	3.900	4.586	69	
32C2	TYR 100	CE2	TYR 34	3.495	4.347	66	2
	TYR 101	CE2	TYR 51	3.900	4.610	50	
1AFV	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**

1A6W	PHE 46	CE2	PHE 89	3.995	4.880	43	10
	TYR 399	CE1	TR5 98	3.774	4.515	301	
	PHE 407	CE2	TR5 98	3.131	3.720	309	
	TYR 401	CD1	TR5 333	3.993	3.675	68	
	PHE 407	CE1	HIS 335	2.569	3.521	72	
	PHE 406	CD1	TYR 395	3.897	4.078	71	
	TRP 410	CE3	TYR 395	3.948	4.857	10	
	TYR 401	CD2	TYR 402	2.873	3.913	1	
	TYR 399	CE1	PHE 407	3.847	4.220	8	
	PHE 46	CD2	TR5 410	3.814	4.175	364	
1L7T	PHE 67	CE2	TYR 91	3.820	4.842	24	2
	PHE 144	CD2	HIS 203	3.648	4.453	59	
1UM5	TYR 34	CD1	TYR 93	3.497	4.352	59	9
	TYR 51	CD2	TYR 96	3.553	4.042	45	
	TYR 175	CE1	TYR 142	3.732	4.595	33	
	TYR 188	CD1	TYR 194	3.593	3.992	6	
	PHE 141	CD2	TYR 200	3.471	4.177	59	
	TYR 194	CD1	PHE 211	3.737	4.417	17	
	TYR 96	CD2	TR5 48	3.189	3.982	48	
	TYR 51	CD2	TYR 96	3.553	4.042	45	
	TYR 182	CE2	PHE 152	3.937	4.887	30	
1JPT.	TYR 50	CD1	TYR 32	3.991	4.298	18	1
1KEN	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
1MCI	TYR 93	CD2	TYR 32	2.585	3.527	61	6
	PHE 101	CE2	TYR 38	3.551	4.186	63	
	TYR 93	CD1	PHE 99	3.061	4.099	6	
	TYR 195	CD2	TR5 189	3.526	4.390	6	
	TRP 18	CE3	TYR 195	3.942	4.538	177	
	PHE 99	CE2	TYR 43	3.925	4.996	34	
1A8J	TYR 93	CD2	TYR 32	2.624	3.668	61	7
	TYR 93	CD1	PHE 99	3.189	4.151	6	
	TYR 176	CE1	TYR 144	3.765	4.765	32	
	TYR 195	CE1	HIS 192	3.302	3.858	3	
	TYR 93	CD1	PHE 99	3.189	4.151	6	
	TRP 189	CE3	TYR 195	3.746	4.602	6	
	PHE 143	CD2	HIS 201	3.372	4.223	58	
2MPA.	TYR 37	CE2	HIS 31	3.422	4.418	6	3
	PHE 101	CD1	TYR 54	3.570	4.449	47	
	TYR 32	CE2	TYR 100	2.561	3.613	68	
1E6O	PHE 107	CE2	TYR 35	2.653	3.623	72	6
	TYR 35	CE2	TR5 110	3.940	4.773	75	
	TYR 48	CD1	HIS 33	3.823	4.903	15	
	TYR 85	CE2	TR5 46	3.219	4.253	39	
	PHE 61	CE2	TYR 85	3.901	4.894	24	
	TYR 31	CD1	TR5 90	2.719	3.575	59	
1MFB	TYR 277	CD2	TYR 282	3.698	4.255	5	2
	TYR 302	CD1	TR5 283	3.382	4.002	20	
1MCK.	PHE 101	CE2	TYR 38	3.146	4.180	63	4
	TYR 93	CD1	PHE 99	2.931	3.941	6	
	TYR 195	CE1	HIS 192	3.103	3.751	3	
	PHE 143	CD2	HIS 201	3.704	4.124	58	
1MCP	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
1L6X	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
1H3U	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**	**nil**
2GJ7	TRP 313	CE3	TYR 319	3.836	4.693	6	2
	TRP 381	CD1	TYR 391	2.973	3.983	10	

This classification enables us to evaluate the contribution of short-, medium-, and long-range contacts in the formation of C–H….π interactions. The sequential distance between residues that contributed to C–H….π interactions were calculated and results are depicted in (Fig.6), 60%, 30%, and 10% of inter-residue C–H….π interactions were found to be long, medium, and short-range interactions, respectively. Long-range C–H….π, these results indicate that long-range C–H...π interactions contribute significantly to the global conformational stability of 'Immunoglobulin proteins'. The role of short and medium range interactions is minimal although they play an important role in the formation of ion pairs[41, 42].

Stabilization centers

We used the SCide server for computing the stabilization centers in the Immunoglobulin proteins data set. We found that 30% of the amino acid residues that contribute donor atoms to C–H...π interactions had one or more stabilization centers in addition to their contribution to C–H...π interactions and similarly 60% of the amino acid residues that contribute acceptor atoms to C–H...π interactions had one or more stabilization centers in addition to their contribution to C–H...π interactions. This result is shown in (Fig.7). From this we infer that, these residues might contribute additional stability to the Immunoglobulin Proteins in addition to their participation in C–H…π interactions.

Conservation score

We used the Consurf server to compute the conservation score of amino acid residues involved in C–H….π interactions in 'Immunoglobulin proteins' and the result is shown in (Fig.8), 29 %of the amino acid residues that contributed donor atoms in C–H….π interactions had the highest conservation score of 9, while as acceptor residues in the range of 9 is 26%, 52% of the amino acid residues of acceptor atom had a conservation score, in the range of 6–8, while donor atom in the range of 6-8 is 46%, and the

contribution of donor and acceptor amino acid residues in the range of 1-5 is 31%and 33%.From these observations, we were able to infer that most of the amino acid residues involved in C–H….π interactions might be conserved in Immunoglobulin proteins. This high conservation of amino acid residues may in some cases is linked to their involvement in C–H….π interactions and to the stability or the function of the protein[18].

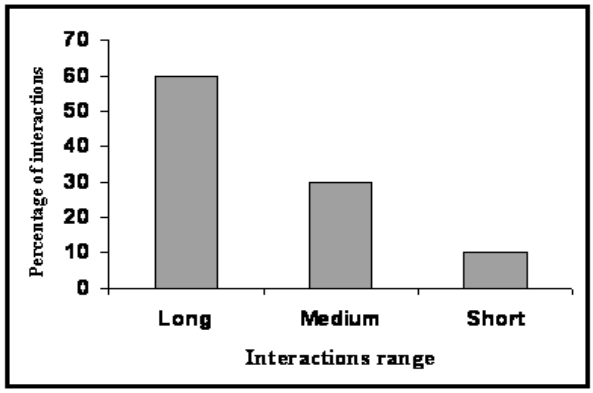

Fig.6: C–H….π interaction range in Immunoglobulin Proteins

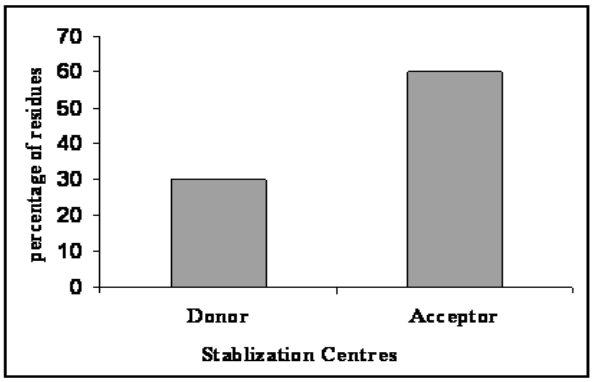

Fig. 7: Stabilization centers in immunoglobulin proteins.

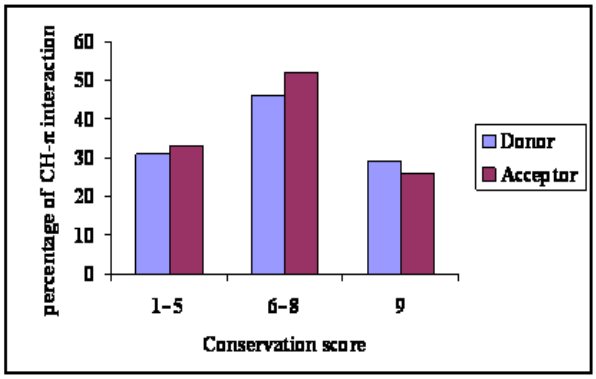

Fig. 8: Conservation score for C–H….π interacting residues in immunoglobulin proteins.

CONCLUSION

In the view of the above analysis, we conclude that the C-H….π interaction is a dominant factor for maintaining structural stability of Immunoglobulin Proteins. The most prominent representatives are the interactions between side-chain to side-chain C-H….π interactions. The geometric parameters calculated for these interactions suggest that C–H….π interactions can be classified as weak H bonds and occur mainly in the distances greater than 4.01 to 4.25Å from the C and H atoms, respectively, in the data set. C–H….π interactions involving aromatic π systems as a donor or acceptor groups are generally found closer to the center of the protein and hence is buried in nature. The secondary structure preference analysis of C–H….π interacting residues showed that the amino acid residues such as Tyr, Phe, Trp and His preferred to be in β Strand confirmation. The sequential distance between residues that contributed donor and acceptor atoms confirmed that long-range C–H….π interactions contribute significantly to the global conformational stability of 'Immunoglobulin proteins'. Significant percentage of both donor and acceptor residues involved in C–H….π interactions had one or more stabilization centers. From this we infer that, these residues might contribute additional stability to the 'Immunoglobulin proteins' in addition to their participation in C–H….π interactions. 78% of the donor amino acid residues and 70% of the acceptor amino acid residues had a higher conservation score. It might be due to their involvement in C–H….π interactions and contribute significantly to the stability of 'Immunoglobulin proteins'. Though weak (around 1 kcal mol-1 for a one unit interaction), a unique feature of this force is that many CH groups may participate simultaneously in the interaction with a π base. Total energy of the interaction will increase by organizing CHs and/or π groups into a favorable chemical structure. Albeit weak, they are numerous and therefore might help explain the well-known problem that protein stabilities, interaction energies and folding energies cannot be calculated very accurately. The consideration of these important interactions might enhance the usefulness of these calculations in general, and further our understanding of protein structures and their functions.

ACKNOWLEDGMENT

The authors thank the management of Vellore Institute of Technology for providing the facilities to carry out this work.

REFERENCES

1. Gromiha MM, Thomas S, and Santhosh C. Role of cation-pi interactions to the stability of thermophilic proteins. Prep. Biochem.Biotech.2002; 32: 355–362.
2. Chakravarty S, Varadarajan R. Elucidation of factors responsible for enhanced thermal stability of proteins: A structural genomics based study.Biochemistry.2002; 41: 8152-8161.
3. Shi Z, Olson CA, Kallenbach NR. Cation-pi interaction in model alpha-helical peptides. J. A., Chem. Soc.2002; 124: 3284–3291.
4. Burghardt TP, N. Juranic S, Macura, Ajtai K. Cation–π interaction in a folded polypeptide. Biopolymers, 2002; 63**:** 261–272.
5. Mulhern TD, Lopez AF, Andrea 'D RJ, Gaunt C, Vandeleur L, Vadas MA, Booker GW, Bagley CJ. Peptide insertions in domain 4 of $h\beta_c$, the shared signaling receptor subunit for gm-csf, il3 and il5, induce ligand-independent activation. J. Mol. Biol, 2000; 297**:** 989–1001.
6. Gromiha MM. Distinct roles of conventional non-covalent and cation–π interactions in protein stability. Biophys. Chem. 2003; 103: 251–258.
7. Harpaz Y, Chothia C, Members of the immunoglobulin superfamily in bacteria. J. Mol. Biol. 1994; 238 (4): 528–39.
8. Barclay A. Membrane proteins with immunoglobulin-like domains--a master super family of interaction molecules. Semin, Immunol. 2003**;** 15 (4): 215–23.
9. Gallivan JP, Dougherty DA. Cation–π interactions in structural biology. Proc.Natl. Acad. Sci. USA. 1999; 96: 9459–9464.
10. Dill KA. Dominant forces in protein folding, Perspect. Biochem. 1990; 29: 7133–7155.
11. Tamres M. Aromatic Compounds as Donor Molecules in Hydrogen Bonding. Journal of the American Chemical Society. 1952; 74: 3375–3378.
12. Reeves LW, Schneider WG. Nuclear magnetic resonance measurements of complexes of chloroform with aromatic molecules and olefins. Canadian Journal of Chemistry. 1957; 35: 251–261.
13. 13 Nishio M, Hirota M, Umezawa Y. (Eds.). The CH/π Interaction, Wiley-VCH, New York, 1998.

14. Matsui I, Matsui E, Sakai Y, Kikuchi H, Kawarabayasi Y, Ura H, Kawaguchi S, Kuramitsu S, Harata K. The molecular structure of hyperthermostable aromatic amino transferase with novel substrate specificity from Pyrococcus horikoshii. The Journal of Biological Chemistry. 2000; 275: 4871–4879
15. Chakrabarti P, Samanta U. CH/π interaction in the packing of the adenine ring in protein structures. Journal of Molecular Biology. 1995; 251: 9–14.
16. Umezawa Y, Nishio M. CH/π interactions as demonstrated in the crystal structure of guanine nucleotide binding proteins, Src homology- 2 domains and human growth hormone in complex with their specific ligands. Bioorganic & Medicinal Chemistry. 1986; 493–504.
17. Muraki M, Harata K, Sugita N, Sato K I. Protein-carbohydrate interactions in human lysozyme probed by combining site-directed mutagenesis and affinity labeling. Biochemistry. 2000; 39: 292–299.
18. Jabs A, Weiss MS, Hilgenfeld R. CH/π interactions in the crystal structure of class I MHC antigens and their complexes with peptides. Journal of Molecular Biology. 1999; 286: 291–304.
19. Shimohigashi Y, Maeda I, Nose T, Ikesue K, Sakamoto H, Ogawa Y. et al. Chymotrypsin Inhibitory conformation induced by amino acid side-chain-side-chain intermolecular CH/π Interaction. Journal of the Chemical Society. 1996; 1: 2479–2485.
20. Shimohigashi Y, Nose T, Yamauchi Y, Maeda I. Design of serine protease inhibitors with conformation restricted by amino acid side-chain side-chain CH/π interaction. Biopolymers. 1999; 51: 9–17.
21. Steiner T. The hydrogen bond in the solid state. Angewandte Chemie International Edition in English. 2002; 41: 48–76.
22. Steiner T, Koellner GJ. Hydrogen bonds with π-acceptors in proteins frequencies and role in stabilizing local 3D structures. Journal of Molecular Biology. 2001; 305: 535-557.
23. Meyer EA, Castellano RK, Diederich F. Interactions with aromatic rings in chemical and biological recognition. Angewandte Chemie International Edition in English. 2003; 42: 1210–1250.
24. 24Tayubi. IA, Sethumadhavan R. Nature of cation-π interactions and its role in structural stability in Immunoglobulin proteins. Biochemistry .Moscow. 2010; 75 (7): 1-1.
25. Berman HM, Westbrook J,. Feng Z, *et al.* The Protein Data Bank. Nucleic Acids Res. 2000; 28**:** 235–242.
26. Tiwari A, Panigrahi SK. HBAT: A complete package for analyzing strong and weak hydrogen bonds in macromolecular crystal structures. In Silico Biology. 2007; 7: 0057.
27. Babu MM. NCI: a server to identify non-canonical interactions in protein Structures. Nucleic Acids Res. 2003; 31(13):3345-8.
28. Ahmad S, Gromiha MM, Fawareh H, Sarai A. ASA View. Database and tool for solvent accessibility representation in proteins. BMC Bioinformatics. 2004; 5: 51
29. Gilis D, Rooman M. Stability changes upon mutation of solvent accessible residues in proteins evaluated by database-derived potentials. Journal of Molecular Biology. 1996; 257: 1112–1126.
30. Gilis D, Rooman M. Predicting protein stability changes upon mutation using database derived potentials: solvent accessibility determines the importance of local versus non local interactions along the sequence. Journal of Molecular Biology. 1997; 272: 276–290.
31. Gromiha MM Selvaraj S. Influence of medium and long range interactions in different structural classes of globular proteins. Journal of Biological Physics. 1997; 23:151162.
32. Gromiha MM, Santhosh C, Ahmad S. Structural analysis of cation-π interactions in DNA binding proteins. International Journal of Biological Macromolecules. 2004; 34: 203–211.
33. Selvaraj S, Gromiha MM. Role of hydrophobic clusters and long range contact networks in the folding of (alpha/beta) 8 barrel proteins. Biophysical Journal. 2003; 84:1919–1925.
34. Gromiha MM, Selvaraj S. Inter-residue interactions in protein folding and stability.
35. Progress. Biophysics and Molecular Biology. 2004; 86: 235–277.
36. Dosztanyi ZS, Fiser A, Simon I. Stabilization centers: J .Mol Biol. 1997; 272: 597-612.
37. Dosztanyi ZS, Magyar CS, Tusnady E, Simon I. SCide: Identification of stabilization centers in proteins. Bioinformatics. 2003; 19: 899-900.
38. Glaser F, Pupko T, Paz I, Bell RE, Bechor D, Martz E, et al. Consurf. Identification of functional regions in proteins by surface mapping of phylogenetic information. Bioinformatics. 2003; 19: 163–164.
39. Boeckman B, Bairoch A, Apweiler R, Blatter MC, Estreicher A, Gasteiger E, et al. The Swiss prot protein knowledge base and its supplement TrEMBL. Nucleic Acids Research. 2003; 31:365–370.
40. Kabsch W, Sander C. Dictionary of protein secondary structure: Pattern recognition of hydrogen-bonded and geometrical features. Biopolymers. 1983; 22: 2577–2637.
41. Brandl M, Weiss MS, Jabs A, Sühnel J, Hilgenfeld R. C-H... -interactions in proteins. Journal of Molecular Biology. 2001; 307: 357–377.
42. Barlow DJ, Thornton JM. Ion-pairs in proteins. J. Mol. Biol. 1983; 168: 867–885.
43. Matthews BW. Structural and Genetic Analysis of Protein Stability. Ann. Rev. Biochem. 1993; 62:139–160.