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EXPLORATION OF CATION-Π INTERACTIONS IN THE STRUCTURAL STABILITY OF FOOD ALLERGENS

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ABSTRACT

Food allergens are components of food and cause various abnormal symptoms. Conservation of three dimensional structure of food allergens are also maintained by conventional as well as non-conventional interactions. Among the non-conventional interactions cation–π interactions play an important role in the stability of protein structures. In this work, we have analyzed the influence of cation–π interactions in the structural stability of food allergens. We observed 67.65% of food allergens (23 out of 34) form one or more cation–π interactions. The chicken ovalbumin has the maximum number of 24 cation–π interactions in its structure. Arg 77 and Trp 19 in maize allergen β-expansin 1b form strongest cation–π interaction. Analysis of the preferred secondary structural conformation for the residues involved in cation–π interaction indicates that the cationic residues preferred to reside in β-strands. Only 25% of the cation-π interactions forming residues in food allergens are conserved. The results obtained in the present study are useful in understanding the contribution of cation–π interactions to the structural stability of food allergens.

Keywords: Cation– π interactions, Food Allergens, Secondary Structure, Accessible surface area.

INTRODUCTION

Food allergens are components of food that trigger body's immune response and causes various abnormal symptoms. This process is referred to as food allergy. Food allergy can cause one or more symptoms that include angioedema, oral allergic syndrome, urticaria, abdominal pain, diarrhea , nausea, vomiting, itching and even life-threatening reactions, such as anaphylactic shock (Ciardiello *et.al.,* 2014). Almost all food allergens are proteins that react with IgE antibodies, induce allergenic sensitization or allergenic reactions (Sánchez, 2005). Though there are multiple characteristics that may be responsible for allergenicity of food allergens, there are no rules to predict the potential allergenicity of these allergens. According to the guidelines of the Codex Alimentarius Food Standard Programme, only a combination of different *in vivo*, *in vitro* and *in silico* methods, that evaluate different aspects of the sensitization and the development of clinical symptoms, can be used to identify the allergenic risk of food proteins (Sánchez, 2005). An *in silico* approach was used to develop and apply novel means of assessing conserved surface features important for IgE cross-reactivity. This structural bioinformatics analysis shows that conservation of three dimensional structure should be included in any assessment of potential IgE cross reactivity (Jenkins et.al., 2005). The unique conservation of three dimensional structures are determined by the interactions of amino acid residues along the polypeptide chain as well as with the surrounding medium. Therefore the study of protein interactions has been vital to the understanding of how proteins function within the cell. In any protein, both the covalent and non-covalent forces determine the three dimensional structure. The various non-covalent interactions that influence the protein structural stability are electrostatic interactions, hydrogen bonding and hydrophobic forces (Voet, 1995). In addition, cation-π interactions between amino acid side-chains are increasingly being recognized as important structural and functional features of proteins and other biomolecules (Gallivan, 1999). Cation-π interactions can occur between cationic side-chain of either lysine or arginine and the aromatic side-chain of phenylalanine, tyrosine or tryptophan. A growing number of experimental and theoretical studies have emphasized the existence of favorable interactions between positively charged groups and π-aromatic systems (Scrutton, 1996, Ma, 1997 and Pletneva *et.al.,* 2001). This type of non-covalent binding force is assumed to be significant in protein structure (Gallivan, 2000) as well as in biomolecular association processes such as antigen–antibody binding (Novotny *et.al.,* 1989 and Pellequer et.al., 2000) and receptor–ligand

interaction (Dougherty, 1990 and Mecozzi, 1996). There are reports of this interaction for their role in the enhancement of stability of thermophilic proteins (Chakravarty S and Varadarajan R, 2002 and Gromiha *et.al.,* 2002), folding of polypeptides (Shi, 2002), and the stability of membrane protein (Gromiha, 2003). The stability and specificity of both protein-DNA (Gromiha *et.al.,* 2004a, 2004b) TIM-barrel proteins (Chakkaravarthi et.al., 2006) and protein-RNA (Chakkaravarthi, 2006 and Anbarasu *et.al.,* 2007) complexes are also reported on the basis of these cation-π interactions.

In this study we have analyzed the cation-π interactions in 34 food allergen proteins. The energetic contribution due to cation-π interactions (Arg-Phe, Arg-Tyr, Arg-Trp, Lys-Phe, Lys-Tyr and Lys-Trp) have been brought out for all of the considered food allergens that form cation-π interactions. The percentage composition of specific amino acid residues contributing to cation-π interactions was calculated. Further, the characteristic features of residues involved in cation-π interactions have been evaluated in terms of secondary structure, solvent accessibility and sequential separation of residues involved in cation- π interaction. The cation-π interaction energy for the pairs with Arg is stronger than that with Lys. Sequential separation of cation-π interactions in food allergen proteins shows that most of the interactions are formed due to long range interactions. Cation–π interaction forming cationic residues Lys and Arg both prefer to be in β-strands, whereas aromatic residues Phe, Tyr and Trp prefer β-strands, α-helix and coil regions respectively.

MATERIALS AND METHODS

DATA SET

The Protein Data Bank Identification code (PBD ID) for the food allergens were collected form Structural Database of Allergenic Proteins (SDAP). SDAP is a web server that provides rapid, cross-referenced access to the sequences, structures and IgE epitopes of allergenic proteins (Ivanciuc, 2003). According to the last updated information date on February 2013, there are 92 allergens in SDAP. Among these allergens 34 allergens were found to be as food allergens (Table 1). Though there are many PDB IDs associated with each of these allergens only the PDB IDs with higher X-ray resolution structure were considered for this study.

Table 1 Food allergens data set

S. No.	SDAP Code	Allergen description	Allergens source	PDB Code
1	Act d 1	Actinidin	Kiwi fruit	2ACT
2	Api g 1	Api g 1 (Pathogenesis related protein PR10)	Celery	2BK0
3	Ara h 1	Ara h 1 – A major peanut allergen	Peanut	3S7I
4	Ara h 2	Ara h 2 – A major peanut allergen	Peanut	3OB4
5	Ara h 3	Ara h 3 – A major peanut allergen	Peanut	3C3V
6	Ara h 6	Ara h 6 – A minor peanut allergen	Peanut	1W2Q
7	Bos d 4	Alpha-lactalbumin; Lactose synthase B protein	Domestic cattle	2G4N
8	Bos d 5	Bovine beta-lactoglobulin	Domestic cattle	1BEB
9	Bra n 1	2S albumin; Calcium-binding pollen allergen	Rapeseed	1PNB
10	Car p 1	Papain	Papaya	2CIO
11	Cyp c 1	Parvalbumin	Common carp	4CPV
12	Dau c 1	Dau c 1z – A major carrot allergen	Carrot	2WQL
13	Fra a 1E	Pathogenesis-related protein, PR-10	Strawberry	2LPX
14	Gal d 2	Ovalbumin	Chicken	1UHG
15	Gal d 3	Ovotransferrin	Chicken	1IEJ
16	Gal d 4	Lysozyme	Chicken	1LKS
17	Gly m 4	Birch pollen allergen	Soybean	2K7H
18	Gly m 6.0101	Glycinin (legumin, 11S globulin), G1 subunit	Soybean	1FXZ
19	Gly m 6.0501	Glycinin (legumin, 11S globulin), G5 subunit	Soybean	2D5F
20	Gly m conglycinin	Conglycinin, 7S seed storage protein	Soybean	1IPK
21	Gly m lectin	Agglutinin	Soybean	1SBF
22	Hor v 1	Barley lipid transfer protein	Barley	1BE2
23	Mal d 2	Thaumatin	Apple	3ZS3

		like food allergen		
24	Mus a 4	Thaumatin-like protein	Banana	1Z3Q
25	Mus a 5	Endo-beta-1,3-glucanase	Banana	2CYG
26	Pru av 1	A – Major cherry allergen	Sweet cherry	1E09
27	Pru av 2	Allergenic thaumatin-like protein	Sweet cherry	2AHN
28	Pru du 6	Amandin	Almond	3FZ3
29	Pru p 3	Lipid transfer protein pan-allergens	Peach	2ALG
30	Ric c 1	2S albumin	Castor bean	1PSY
31	Tri a 18	Agglutinin isolectin A	Wheat	2CWG
32	Vig r 6.0101	Cytokinin-specific binding protein	Mung bean	2FLH
33	Zea m 1	beta-expansin 1b	Maize	2HCZ
34	Zea m 14	Lipid transfer protein	Maize	1MZM

COMPUTATION OF AMINO ACID COMPOSITION

The amino acid composition for each amino acid residue that are involved in cation–π interactions (Lys, Arg, Phe, Trp and Tyr) were computed using the standard formula,

$$\mathbf{Comp}\ (i) = n\ (i)\ /N \quad \text{---------- [1]}$$

where $n(i)$ is the number of amino acids of type i and N is the total number of amino acids in a protein.

ESTIMATION OF ENERGETICALLY SIGNIFICANT CATION-II INTERACTIONS

Cation–π interactions are found to be common among structures in the Protein Data Bank (PDB). To search the cation–π interaction in PDB a computer program CAPTURE (Cation–π Trends Using Realistic Electrostatics) is available at http://capture.caltech.edu and was developed to calculate the distance between the cationic group ammonium nitrogen (NZ) in Lys or the guanidinium carbon (CZ) in Arg and the centers of all aromatic rings (Gallivan, 1999). In the present study only energetically significant interactions were considered. The percentage composition of a specific amino acid residue contributing to cation-π interactions is obtained by the equation,

$$\mathbf{comp}_{\mathbf{cat\text{-}\pi}}\ (i) = \mathbf{n}_{\mathbf{cat\text{-}\pi}}\ (i)\ \mathbf{x\ 100/n}(i), \quad \text{---------- [2]}$$

where i stands for the five residues, Lys, Arg, Phe, Trp and Tyr, $n_{cat-\pi}$ is the number of residues involved in cation- π interactions and n (i) is the number of residues of type i in protein structures.

We have computed the energetic contribution of cation-π interactions for each food allergen in the data set and for all possible pairs of positively charged aromatic amino acids. The total cation-π interaction energy ($E_{cat-\pi}$) has been divided into electrostatic (E_{es}) and van der Waals energy (E_{vw}) and were computed using the program CAPTURE, which has implemented a subset of OPLS force field (24) to calculate the energies. The electrostatic energy (E_{es}) is calculated using the equation

$$E_{es} = q_i q_j e^2 / r_{ij} \quad \text{---------- [3]}$$

where q_i and q_j are the charges for the atoms i and j respectively, and r_{ij} is the distance between them. The van der Waals energy is given by

$$E_{vw} = 4\varepsilon_{ij}\left[(\sigma_{ij}^{12}/r_{ij}^{12}) - (\sigma_{ij}^{6}/r_{ij}^{6})\right] \quad \text{---------- [4]}$$

Where $\sigma_{ij} = (\sigma_{ii}\ \sigma_{jj})^{1/2}$ and $\varepsilon_{ij} = (\varepsilon_{ii}\ \varepsilon_{jj})^{1/2}$; σ and ε are the van der Waals radius and well depth, respectively.

SECONDARY STRUCTURE AND SOLVENT ACCESSIBILITY PREFERENCE OF CATION-II INTERACTION FORMING RESIDUES

Secondary structure preference and solvent accessibility of the amino acid residues are among the key factors that are essential to understand the structure-function relationship of proteins. Secondary structure and accessibility for the amino acid residues were calculated using Definition of Secondary Structure of Protein (DSSP) (Kabsch, 1983). In the study, the preference pattern of the amino acid residues involved in each type of the cation–π interaction in a particular secondary structure and their solvent accessibility were systematically analysed. The secondary structural units have been classified as helix, strand, turn and coil in accordance with (Heringa, 1999). Solvent accessibility or accessible surface area (ASA) is defined as the protein surface that is in contact with solvent. It is measured by the set of points occupied by the center of a water molecule that is being rolled along the van der Waals surface of the protein molecule (Kumarevel et.al., 1998). The ASA for each amino acid in a protein was calculated using the ASA-View program (Ahmad, 2004) which is available at http://www.abren.net/asaview/ and the ASA was divided into 3 classes, 0-20%, 20-50% and >50%, indicating respectively the least, moderate and high accessibility of the amino acid residues to the solvent (Gilis, 1996, 1997). The percentage of an amino acid in a particular ASA class involved in a particular non-canonical interaction was evaluated using the relation

$$\%\ \mathbf{ASA}_{\mathbf{AA}} = (\mathbf{N}_{\mathbf{AA}}\ \mathbf{cation\text{-}\pi}\ /\ \mathbf{N}_{\mathbf{AA}})\ \mathbf{x\ 100} \quad \text{----------[5]}$$

Where N_{AA} cation-π and N_{AA} indicates the number of instances a particular amino acid belonging to a specific ASA class is involved in cation-π and the total number of instance of the same amino acid (found in that ASA class) in the whole data set respectively.

SEQUENTIAL SEPARATION OF AMINO ACID RESIDUES

The amino acid residues involved in the cation-π were classified as short range (<±3 residues), medium range (±3 or ±4 residues) and long range (>±4 residues) based on their location in the amino acid sequence (31, 32). This classification enabled us to evaluate the contribution of long-range contacts in the formation of cation–π interactions.

CONSERVATION OF AMINO ACID RESIDUES

Conservation of residues in each food allergens has been evaluated with the aid of the Consurf server and has the web link http://consurf.tau.ac.il/ (33). This server compares the sequence of a PDB chain with the proteins deposited in Swiss-Prot (34) and finds the ones that are homologous to the PDB sequence. These protein sequence alignments were used to classify the residues in each food allergens into 9 categories: from very variable (score = 1) to highly conserve (score = 9).

IDENTIFICATION OF STABILIZING RESIDUES

We have identified stabilizing residues in each food allergen protein with the aid of the SRide server, which is available at http:\\sride.enzim.hu (35, 36). This server computes the different measures of stability such as surrounding hydrophobicity (H_p), long range order (LRO), stabilization center (SC) and conservation of residues. The stabilization residues in food allergen proteins have been delineated with certain cutoff values for each term (i.e., the stabilizing residues is the one in which the values for all these four parameters are equal to or greater than the specified cutoff values). In this study, we have used the following conditions to predict the stabilizing residues: (i) $H_p \geq 20$ kcal/mol; (ii) LRO ≥ 0.02; (iii) SC ≥ 1; and (iv) conservation score ≥ 6.

RESULTS

This study mainly focused on the analysis of cation-π interactions in the structural stability of food allergens; among the 34 food allergens identified through SDAP, only 23 food allergens have energetically significant cation-π interactions. The subsequent analysis of cation-π interactions were carried out only in those 23 food allergens.

Relationship between the total number of amino acid residues and number of cation-π interactions in food allergens were found to have a positive correlation between the number of residues and number of cation–π interactions with the correlation coefficient is 0.52 as shown in Figure 1. The composition of amino acid residues that are involved in cation–π interactions was analysed and the results for food allergens along with globular proteins are presented in Table 2. Lys and Phe have the highest occurrence of cationic and aromatic amino acids involved in cation–π interactions of food allergens. The number of cation–π interactions in each of the food allergens and their energetic contributions are presented in Table 3. As we considered only the proteins that have cation–π interactions we observed an average of 5 cation–π interactions in food allergens.

Table 2- Composition of aromatic and positively charged residues in food allergens

Proteins	Lys%	Arg%	Phe%	Tyr%	Trp%
Food allergens	5.97 ± 3.36	3.80 ± 2.51	4.33 ± 1.86	3.82 ± 1.82	1.35 ± 1.17
Globular proteins	5.83	4.74	3.97	3.60	1.48

Table 3-Cation- π interaction energetic contribution in food allergens

PDB code	$N_{cat-\pi}$	$-E_{es}$	$-E_{vw}$	$-E_{cat-\pi}$
1BEB	3	7.29	7.02	14.31
1FXZ	8	14.09	11.44	25.53
1IEJA	7	24.70	9.01	33.71
1IPK	10	21.60	16.52	38.12
1LKSA	2	5.42	7.05	12.47
1UHG	24	83.98	50.09	134.07
1Z3QA	2	7.90	5.15	13.05
2ACTA	3	12.69	6.12	18.81
2AHN	1	4.75	0.89	5.64
2CIOA	6	20.82	9.70	30.52
2CWG	5	10.93	15.62	26.55
2CYZA	3	5.25	7.23	12.48
2FLH	4	17.01	4.41	21.42
2G4N	5	12.56	4.72	17.28
2HCZX	6	18.66	3.99	22.65
2K7HA	1	5.62	+ 4.05	1.57
2LPXA	1	2.69	0.95	3.64
3C3VA	6	15.87	14.80	30.67
3FZ3	4	4.99	5.04	10.03
3OB4A	3	11.67	5.49	17.16
3S7I	7	17.16	12.54	29.70
3ZS3A	2	3.07	3.16	6.23
4CPVA	1	4.88	1.26	6.14
Average	5.00±4.84	14.50±16.48	8.62±10.36	23.12±26.29

Occurrence of cation- π interactions in more than one chain of the PDB id is bolded.

$N_{cat-\pi}$, number of cation-π interactions in a protein. E_{es}, E_{vw}, $E_{cat-\pi}$ are, respectively, electrostatic, van der Waals and total cation-π interaction energy.

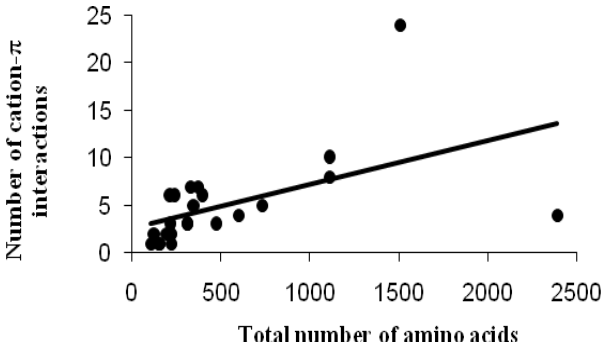

Figure 1- Relationship between the total number of amino acid residues and number of cation-π interactions in food allergens (coefficient of correlation = 0.52).

The energetic contribution of each cationic-aromatic pairs of amino acids in food allergens has been computed and the results are presented in Table 4. The number of residues involved in cation–π interactions were, 33, 81, 31, 55 and 28 for Lys, Arg, Phe, Tyr and Trp,

respectively. We found that 67.65% of the food allergens (23/34) form one or more cation- π interactions and few residues form cation–π interactions with several other residues (eg. K209 in chicken ovotransferin (1EIJ), R43 in thaumatin like protein of banana (1Z3Q) etc.). The strongest contribution is observed for the interaction between Arg77 and Trp19 in the X chain of β-expansin 1b in maize (2hcz) and the cation–π interaction energy is –11.03 kcal/mol. The frequency of cation–π interaction pairs at different intervals of energy is plotted in Figure 2. We observed that most of the cation–π interactions have the energy in the range of –3 to –4 kcal/mol. The average cation–π interaction energy for all the six possible pairs between cationic and aromatic residues in food allergens is tabulated in Table 5. We observed that in food allergens, Arg-Trp pair has the strongest contribution among all pairs.

Table 4- Cation-π interaction energy in food allergens

PDB code	R-F (-kcal/mole)	R-Y (-kcal/mole)	R-W (-kcal/mole)	K-F (-kcal/mole)	K-Y (-kcal/mole)	K-W (kcal/mole)
1BEB A		R124 –Y19 (5.86)			K14 –Y99 (2.90)	
1BEB B		R124 –Y19 (5.55)				
1FXZ A		R162-Y164 (3.00)	R61-W131 (4.72)		K113-Y115 (2.19) K175-Y176 (2.17)	
1FXZ B		R162-Y164 (2.55)	R61-W131 (3.62)			
1FXZ C		R162-Y164 (2.77)	R61-W131 (4.51)			
1IEJ A	R233-F104 (4.40)	R121-Y191 (3.27)	R309-W264 (5.91)	K112-F104 (5.84)	K209-Y82 (6.16) K209-Y92 (4.17) K301-Y92 (7.16)	
1IPKA	R31-F19 (4.81) R39-F13 (3.07) R220-F234 (4.56) R347-F233 (3.41)			K124-F71 (2.65)		
1IPKC	R31-F19 (4.00) R39-F13 (4.69) R220-F234 (3.68) R347-F233 (3.49)			K124-F71 (3.76)		
1LKSA	R114-F34 (4.92)		R73-W62 (7.55)			
1UHGA		R50-Y42 (+2.66) R104-Y106 (3.31) R359-Y281 (7.81)	R104-W148 (10.03)	K186-F217 (2.72)		
1UHGB		R50-Y42 (8.34) R104-Y106 (3.69) R110-Y111 (6.54) R359-Y281 (7.89)	R104-W148 (9.87)	K186-F217 (2.95)		
1UHGC		R50-Y42 (8.13) R84-Y125 (3.13) R104-Y106 (3.65) R359-Y281 (7.67)	R104-W148 (8.57)	K186-F217 (2.91)		
1UHGD		R50-Y42 (7.70) R84-Y125 (2.75) R104-Y106 (3.67) R110-Y111 (4.78) R359-Y281 (7.22)	R104-W148 (9.90)	K186-F217 (3.47)		
1Z3QA		R43-Y74(8.47)	R43-W45 (4.58)			
2ACTA		R8-Y193 (7.11) R195-Y177 (4,59)	R198-W178 (7.11)			
2AHN				K128-F208 (5.64)		
2CIOA		R8-Y186 (6.30) R96-Y94 (4.31) R188-Y170 (5.24)	R59-W69 (4.35)		K100-Y86 (7.77)	K10-W7(5.55)

2CWGA		R2-Y23 (6.17) R45-Y66 (5.39)				K149-W150 (3.24)
2CWGB		R2-Y23 (4.04) R45-Y66 (7.71)				
2CYGA	R298-F233 (3.16) R299-F247 (5.28)	R115-Y116 (4.-04)				
2FLHA					K32-Y149 (6.06)	
2FLHB					K32-Y149 (5.02)	
2FLHC					K32-Y149 (3.42)	
2FLHD					K32-Y149 (6.92)	
2G4ND					K93-Y18 (2.83)	
2G4NE					K93-Y18 (3.46)	K5-W118 (3.54)
2G4NF					K93-Y18 (3.64)	K5-W118 (3.81)
2HCZX		R139-Y15 (4.03)	R77-W19 (11.03) R126-W26 (2.87) R136-W19 (+5.69) R213-W187 (7.08)		K166-Y167 (3.33)	
2K7HA				K54-F65 (1.57)		
2LPXA					K56-Y67 (3.64)	
3C3VA	R81-F84 (5.78) R194-F192 (3.59)	R45-Y52 (8.81) R149-Y100 (5.53) R208-Y209 (3.85)	R388-W389(3.11)			
3FZ3A			R72-W56(2.28)			
3FZ3B			R72-W56(2.28)			
3FZ3E			R72-W56(2.33)			
3FZ3F			R72-W56(2.74)			
3OB4A			R316-W232(7.87)		K170-Y167 (3.22)	K15-W230 (6.07)
3S7IA	R185-F298 (4.71) R194-F182 (2.78) R202-F177 (4.10)	R498-Y500 (6.27)				
3S7IB	R185-F298 (4.39) R194-F182 (3.32) R202-F177 (4.13)					
3ZS3A	R39-F31 (3.18)				K167-Y168 (3.05)	
4CPVA				K96-F57 (6.14)		

The fifth letter of the PDB code indicates the chain.

Table 5- Average energy contribution for each amino acid pair involved in cation-π interaction of food allergens

Amino acid Pair	$-E_{es}$	$-E_{vw}$	$-E_{cat-\pi}$
Arg-Phe	2.11 ± 0.61	1.95 ± 0.58	4.06 ± 0.80
Arg-Tyr	2.78 ± 1.25	2.15 ± 1.69	4.93 ± 2.41
Arg-Trp	3.65 ± 1.44	2.27 ± 2.68	5.92 ± 3.67
Lys-Phe	3.26 ± 1.32	0.21 ± 1.52	3.47 ± 1.40
Lys-Tyr	3.04 ± 1.46	1.06 ± 0.19	4.11 ± 1.53
Lys-Trp	3.53 ± 1.28	0.91 ± 0.18	4.44 ± 1.28

Table 6- Secondary structure, solvent accessibility, conservation score and sequence distance of cation- π interaction forming residues

PDB code	Cation	Residue	Str	ASA	Cons	π	Residue	Str	ASA	Cons	Dseq
1BEBA	Arg Lys	124 14	C H	78 114	9 6	Tyr Tyr	19 99	C C	1 37	9 7	105 85

1BEBB	Arg	124	C	69	9	Tyr	19	C	1	9	105
1FXZA	Arg	162	S	5	1	Tyr	164	S	3	1	2
	Arg	61	S	3	5	Trp	131	S	8	6	70
	Lys	113	C	125	9	Tyr	115	S	42	5	2
	Lys	175	H	69	1	Tyr	176	H	14	1	1
1FXZB	Arg	162	C	2	1	Tyr	164	S	7	1	2
	Arg	61	S	4	5	Trp	131	S	9	6	70
1FXZC	Arg	162	S	1	1	Tyr	164	S	4	1	2
	Arg	61	S	3	5	Trp	131	S	8	6	70
1IEJA	Arg	233	S	48	8	Phe	104	C	16	6	129
	Arg	121	C	33	8	Tyr	191	H	1	8	70
	Arg	309	S	69	4	Trp	264	H	43	1	45
	Lys	112	C	55	6	Phe	104	C	16	6	8
	Lys	209	S	1	8	Tyr	82	S	1	8	127
	Lys	209	S	1	8	Tyr	92	S	0	9	117
	Lys	301	C	4	8	Tyr	92	S	0	9	209
1IPKA	Arg	31	S	24	8	Phe	19	S	30	7	12
	Arg	39	H	96	1	Phe	13	S	0	9	26
	Arg	220	H	87	5	Phe	234	S	15	3	14
	Arg	347	S	13	7	Phe	233	S	8	2	114
	Lys	124	S	9	4	Phe	71	S	7	1	53
1IPKC	Arg	31	S	26	8	Phe	19	S	28	7	12
	Arg	39	H	80	1	Phe	13	S	0	9	26
	Arg	220	H	87	5	Phe	234	S	20	3	14
	Arg	347	S	13	7	Phe	233	S	4	2	114
	Lys	124	S	12	4	Phe	71	S	7	1	53
1LKSA	Arg	114	H	147	1	Phe	34	H	61	1	80
	Arg	73	C	145	2	Trp	62	T	93	1	11
1UHGA	Arg	50	H	59	7	Tyr	42	H	8	1	8
	Arg	104	S	3	6	Tyr	106	S	9	6	2
	Arg	359	S	65	3	Tyr	281	S	34	3	78
	Arg	104	S	3	6	Trp	148	H	4	9	44
	Lys	186	S	62	1	Phe	217	S	6	6	31
1UHGB	Arg	50	H	30	7	Tyr	42	H	9	1	8
	Arg	104	S	2	6	Tyr	106	S	9	6	2
	Arg	110	T	133	6	Tyr	111	C	22	4	1
	Arg	359	S	68	3	Tyr	281	S	24	3	78
	Arg	104	S	2	6	Trp	148	H	8	9	44
	Lys	186	S	65	1	Phe	217	S	7	6	31
1UHGC	Arg	50	H	49	7	Tyr	42	H	10	1	8
	Arg	84	H	23	5	Tyr	125	C	24	8	41
	Arg	104	S	2	6	Tyr	106	S	8	6	2
	Arg	359	S	63	3	Tyr	281	S	35	3	78
	Arg	104	S	2	6	Trp	148	H	4	9	44
	Lys	186	S	46	1	Phe	217	S	9	6	31
1UHGD	Arg	50	H	35	7	Tyr	42	H	12	1	8
	Arg	84	H	33	5	Tyr	125	C	21	8	41
	Arg	110	T	123	6	Tyr	111	C	13	4	1
	Arg	104	S	13	6	Tyr	106	S	12	6	2
	Arg	359	S	76	3	Tyr	281	S	35	3	78
	Arg	104	S	13	6	Trp	148	H	16	6	44
	Lys	186	S	61	1	Phe	217	S	7	1	31
1Z3QA	Arg	43	S	22	8	Tyr	74	C	105	4	31
	Arg	43	S	22	8	Trp	45	S	0	7	2
2ACTA	Arg	8	H	98	9	Tyr	193	S	23	8	185
	Arg	195	S	39	6	Tyr	177	S	17	7	18
	Arg	198	C	13	9	Trp	178	S	0	4	20
2AHN	Lys	128	C	72	9	Phe	208	C	1	4	80
2CIOA	Arg	8	T	97	9	Tyr	186	S	21	9	89
	Arg	96	C	21	1	Tyr	94	C	138	1	2

	Arg	188	S	66	6	Tyr	170	S	42	6	18
	Arg	59	T	137	1	Trp	69	H	94	2	10
	Lys	100	T	62	1	Tyr	86	C	0	9	14
	Lys	10	T	117	6	Trp	7	T	25	8	3
2CWGA	Arg	2	C	64	3	Tyr	23	C	114	3	21
	Arg	45	S	80	7	Tyr	66	S	37	2	21
	Lys	149	T	97	2	Trp	150	T	207	3	1
2CWGB	Arg	2	C	96	3	Tyr	23	C	58	3	21
	Arg	45	S	105	7	Tyr	66	S	100	2	21
2CYGA	Arg	298	C	46	9	Phe	233	S	65	7	65
	Arg	299	C	61	6	Phe	247	H	3	3	52
	Arg	115	S	62	9	Trp	116	S	11	7	1
2FLHA	Lys	32	H	65	8	Tyr	149	H	17	8	117
2FLHB	Lys	32	H	84	8	Tyr	149	H	12	8	117
2FLHC	Lys	32	H	32	8	Tyr	149	H	26	8	117
2FLHD	Lys	32	H	72	8	Tyr	149	H	9	8	117
2G4ND	Lys	93	H	56	9	Tyr	18	H	77	9	75
2G4NE	Lys	93	H	50	9	Tyr	18	H	52	4	75
	Lys	5	H	77	7	Trp	118	S	17	6	113
2G4NF	Lys	93	H	51	9	Tyr	18	H	66	4	75
	Lys	5	H	79	7	Trp	118	H	15	6	113
2HCZX	Arg	139	C	133	4	Tyr	15	C	45	1	124
	Arg	77	S	73	6	Trp	19	C	47	8	58
	Arg	126	T	13	6	Trp	26	S	57	6	100
	Arg	136	S	59	5	Trp	19	C	47	8	117
	Arg	213	S	56	8	Trp	187	C	51	5	26
	Lys	166	S	17	4	Tyr	167	C	33	6	1
2K7HA	Lys	54	S	54	5	Phe	65	S	70	4	11
2LPXA	Lys	56	S	60	5	Tyr	67	S	78	4	11
3C3VA	Arg	81	S	14	6	Phe	84	S	3	5	3
	Arg	194	C	43	9	Phe	192	C	21	4	2
	Arg	45	S	112	8	Tyr	52	S	31	2	7
	Arg	149	S	57	3	Tyr	100	S	33	6	49
	Arg	208	H	132	4	Tyr	209	H	13	3	1
	Arg	388	H	97	5	Trp	389	H	166	1	1
3FZ3A	Arg	72	S	32	9	Trp	56	C	0	8	14
3FZ3B	Arg	72	S	24	9	Trp	56	S	3	8	14
3FZ3E	Arg	72	S	24	9	Trp	56	C	0	8	14
3FZ3F	Arg	72	S	26	9	Trp	56	S	5	8	14
3OB4A	Arg	316	H	56	3	Trp	232	H	2	6	84
	Lys	170	S	100	5	Tyr	167	S	57	6	3
	Lys	15	C	19	6	Trp	230	H	16	3	215
3S7IA	Arg	185	S	75	7	Phe	298	C	8	2	113
	Arg	194	S	28	8	Phe	182	S	13	7	14
	Arg	202	H	63	1	Phe	177	S	0	9	25
	Arg	498	S	58	3	Tyr	500	S	29	6	2
3S7IB	Arg	185	S	74	7	Phe	298	C	7	2	113
	Arg	194	S	26	8	Phe	182	S	0	7	14
	Arg	202	H	64	1	Phe	177	S	0	9	25
3ZS3A	Arg	39	S	107	1	Phe	31	C	2	3	8
	Lys	167	H	102	6	Tyr	168	H	25	7	1
4CPVA	Lys	96	C	68	6	Phe	57	C	65	7	39

Str: secondary structure; H: helix; S: strand; T: turn; C: coil; ASA: accessible surface area or solvent accessibility. The values are in $Å^2$. Cons: conservation score – varies from very variable (score = 1) to highly conserve (score = 9), Dseq: sequence distance of separation between cationic and aromatic residues.

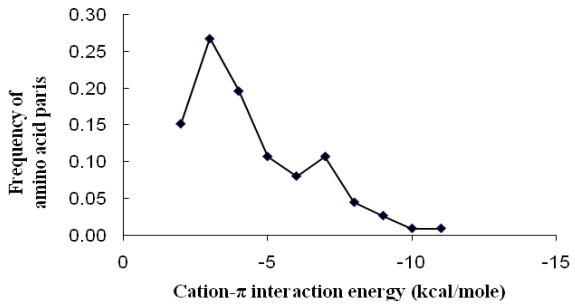

Figure 2- Frequency of amino acid pairs at different ranges of cation-π interaction energy in food allergens

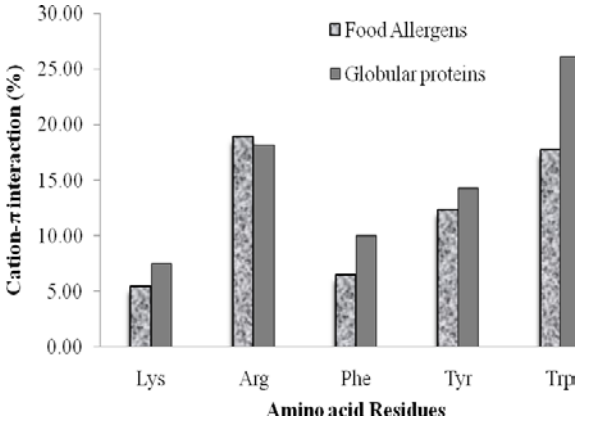

Figure 3- Percentage of aromatic and positively charged residues contributing towards cation- π interactions in food allergens and globular proteins

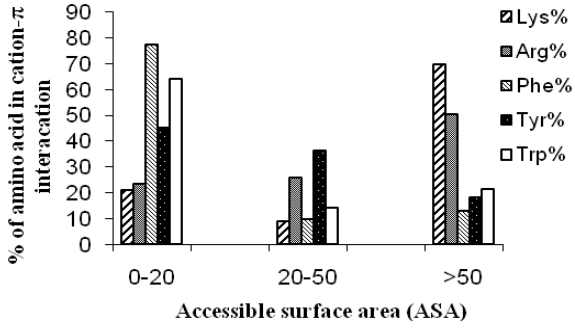

Figure 4- Percentage cation-π interaction amino acid in different range ASA of food allergens

We have estimated the percentage of aromatic and positively charged amino acids that are involved in cation–π interactions in food allergen protein structures. The relative contribution of each of the five amino acid residues in food allergens and globular proteins is depicted in Figure 3. We found that the contribution of aromatic residues in food allergens to form cation–π interactions is (Phe 6.5%, Tyr 12.32% and Trp 17.71%). We have calculated the sequential distance between the cationic and aromatic residues for each of the cation–π interactions and the results are presented in Table 6. We found that in food allergens 18%, 3% and 79% of cation–π interactions are influenced by short, medium and long range interactions.

In Table 6, we have also included the conservation score for all cation–π interactions forming residues in food allergens. Interestingly, 14% of the residues have the highest score of 9 and 64% of the residues have the conservation score ≥6. On the other hand, more than 25% of the cation– π interaction forming residues was highly conserved in structural class of proteins.

We have estimated the solvent accessibility of all residues that are involved in cation–π interaction with the aid of DSSP (Kabsch, 1983). We have analyzed the percentage of cation- π interaction forming residues at various range of solvent accessibility, such as: 0-20% (buried), 20-50% (partially buried), and >50% (surface exposed) (Gilis, 1996, Gills, 1997 and Gromiha, 2004) and the results are depticted in Figure 4. The cation–π interaction forming Lys and Arg prefer to be on the surface of food allergens. We have calculated the occurrence of cation–π interaction forming residues in different secondary structures of food allergens and the results are presented in Table 7. We found that in food allergens the cation–π interaction forming Lys and Arg prefers to be in β-strand. Among the cation–π interaction forming aromatic residues, Phe is accommodated in β-strands. On the other hand, cation–π interactions forming Tyr and Trp in food allergens prefer to be in helical segments and coil respectively.

We have identified 280 stabilizing residues in 22 out of the 23 considered food allergens and the results are presented in Table 8. We observed an average of 2.4% residues as stabilizing ones (280 out of 11882) in food allergens.

Table 7- Percentage of cation-π interaction involving residues in secondary structure of food allergens

Secondary structure	Lys %	Arg %	Phe %	Tyr %	Trp %
Helix	36.36 (31.06)	20.99 (23.00)	6.45 (25.37)	27.27 (21.08)	35.17 (31.85)
Coil	18.18 (27.24)	17.28 (25.33)	25.81 (19.90)	25.45 (20.10)	17.86 (22.22)
Strand	36.36 (30.07)	55.56 (38.00)	67.74 (48.26)	47.27 (53.19)	35.71 (34.07)
Turn	9.09 (11.63)	6.17 (13.67)	0.00 (6.47)	0.00 (5.64)	10.71 (11.85)

The frequency of occurrence of each residue in the whole dataset is shown in parenthesis.

Table 8-Stabilizing residues in food allergens

PDB code	Stabilization Residues
1BEB A	Tyr 20, Ala 26, Ser 27, Val 43, Cys 106, Val 123,
1BEB B	Tyr 20, Ser 21, Ala 26, Ser 27, Val 43, Cys 106, Cys 121, Val 123
1FXZ A	Leu 60, Ile 72, Leu 122, Trp 132, Asn 135, Val 352, Ser 361, Ala 365, Val 401
1FXZ B	Leu 60, Trp 132, Asn 135, Val 352, Ser 361, Ala 365, Val 401, Val 413
1FXZ C	Leu 60, Ile 72, Leu 122, Trp 132, Asn 135, Val 352, Ser 361, Ala 365, Val 401, Val 413
1IEJ A	Ala 56, Ile 57, Ala 78, Ala 79, Glu 80, Tyr 93, Ala 94, Val 97, Cys 115, Cys 160, Val 208, Leu 226, Asn 243
1IPKA	Tyr49, Asn59, Thr60, Ile61, Asp70, Val75, Tyr110, Asn113, Ile122, Ile123, His265, Ile271, Val272, Ile273, Val326, Gly339
1IPKC	Arg 50, , Asn59, Thr60, Ile61, Tyr110, Asn113, His265, Ile271, Val272, Ile273, Leu 274, Val326, Gly339
1LKSA	Val 29, Glu 35, Thr 40, Cys 64
1UHGA	Asn 176, Met 211, Ile 278, Val 280, Pro 363, Phe 364, Leu 365, Phe 366, Arg 381
1UHGB	Asn 176, Met 211, Leu 245, Ile 278, Val 280, Phe 364, Leu 365, Phe 366, Arg 381
1UHGC	Asn 176, Met 211, Leu 245, Ile 278, Val 280, Pro 363, Phe 366, Arg 381
1UHGD	Asn 176, Met 211, Ile 278, Val 280, Gly 338, Phe 366, Arg 381
1Z3QA	Arg 43, Asn 79, Ala 82, Ile 97, Met 107, Val 196, Val 197, Phe 198
2ACTA	Ala 27, Ser 49, Pro 132, Ala 136, Ala 163, Gly 168, Tyr 177, Met 194, Ser 213, Pro 215
2AHN	Gly 16, Ala 88, Gly 111, Met 116, Phe 220
2CIOA	Ala 27, Ser 49, Ser 131, Ala 162, Tyr 170, Leu 172, Ile 187, Arg 188, Phe 207, Pro 209
2CWGA	Cys 18, Cys 104, Cys 147
2CWGB	Cys 18, Cys 104
2CYGA	Gly 30, Val 31, Cys 32, Arg 59, Leu, 80, Ala 136, Ser 156, Val 260, Ser 263, Glu 264, Tyr 307, Glu 310, Met 311, Phe 312, Gly 326
2FLHA	Val 38, Lys 39, Phe 58, Val 85, Ser 119, Tyr 120
2FLHB	Val 38, Lys 39, Phe 58, Val 85, Ser 119
2FLHC	Val 38, Lys 39, Phe 58, Arg 68, Val 85, Ser 119, Tyr 120
2FLHD	Val 38, Lys 39, Phe 58, Arg 68, Val 85,Thr 112, Ser 119, Tyr 120
2G4ND	Glu 25, Cys 28, Trp 60, Cys 61
2G4NE	Trp 60, Cys 61, Lys 93
2G4NF	T rp 60, Cys 61, Lys 93
2HCZX	Ala 22, Thr 25, Thr 56, Gly 59, Asn 60, Val 76, Leu 108, Phe 150, Val 163, Met 177, Ser 211, Val 227
2K7HA	Leu 67
2LPXA	Lys 69, Ser 85, Leu 86
3C3VA	Ser 71, Phe 84, Glu 91, Asp 154, Leu 155, Trp 165, Ala 403, Ser 415, Ile 417, Tyr 418, Ala 419, Val 448, Val 467
3FZ3A	Leu 80, Ile 94, Ala 203, Ala 222, Gly 396, Val 441
3FZ3B	Leu 80, Ile 94, Val 441, Phe 489
3FZ3E	Leu 80, Ile 94, Asp 200, Ala 203, Ala 222, Gly 396, Val 441
3FZ3F	Leu 80, Ile 94, Val 441, Ile 470, Phe 489
3OB4A	Asp 58, Pro 257
3S7IA	Leu 224, Leu 443, His 447, Met 453, Val 454, Phe 531, Gly 532
3S7IB	Leu 224,Ala 243, Leu 443, His 447, Val 454, Val 509, Phe 531, Gly 532
3ZS3A	Ala 88, Pro 115, Met 116
4CPVA	Nil

Residues shown in bold are involved in both stabilization and cation-π interaction

DISCUSSION

From the composition analysis of cation-π interaction residues (Table 2), we observed that in food allergens, Phe has the highest occurrence among the aromatic residues, which is similar to globular proteins (Gallivan, 1999). As in globular protein occurrence of Trp in food allergens is lower than the other two aromatic amino acids. Similar to globular proteins (Gallivan, 1999) the number of Lys is higher than Arg in food allergens. It was observed that the composition of cation–π interaction forming residues in food allergens is similar to other globular proteins.

The number of cation–π interactions varies for different food allergens (Table 3); considering all the food allergens, chicken ovalbumin (1uhg) has the maximum number of 24 cation–π interactions whereas allergenic thaumatin-like protein of sweet cherry (2ahn), birch pollen allergen of soybean (2k7h), pathogenesis related protein PR10 of strawberry (2lpx) and parvalbumin in crops (4cpv) have only one cation–π interactions**.** Though the A chain length of actinidin in kiwi fruit (2act) and papain in papaya (2cio) is similar (217 and 218 amino acids) and shares the identity as well as the similarity of 46.6 % and 57.9 % respectively, the number of cation–π interactions varies to 3 and 6, respectively. This reflects there is no correlation between chain length and number of cation–π interactions.

Though the food allergens have the same number of energetically significant cation–π interactions strength of interaction energy varies significantly. For example, A chains of birch pollen allergen of soybean (2k7h), pathogenesis related protein PR10 of strawberry (2lpx) and parvalbumin in crops (4cpv), each having single cation–π interaction, strength of interactions energy varies. However, we found positive correlation between the number of cation–π interactions and their energies (r = 0.97). The composition of cation–π interaction energy into electrostatic and van der Waals energy terms showed that among the 23 out of 34 food allergen proteins that have cation- π interactions, 19 have stronger electrostatic energy than van der Waals energy and an opposite trend is observed for 4 proteins.

The occurrence of cation-π interaction between homologous chains of particular food allergenic protein varies. For example though there are two cation-π interactions that were observed for the A chain of bovine β-lactoglobulin (1beb), the B chain of the same allergen has only one cation-π interaction. Further, the similar kind of cation-π interaction energy between different chains of particular food allergens differs explicitly. The difference in occurrence as well as the energy of cation-π interactions between different chains of same food allergenic protein is due to the variation in the distance as well as the electrostatic and van der Waals interaction energy between residues proposed to be involved in such interactions.

Further, in globular proteins, there is an average of one energetically significant cation–π interaction for every 81 residues **(**Gallivan, 1999**)**. In food allergens, we have identified 114 cation–π interactions among 11,882 amino acid residues, indicating the presence of one cation–π interaction for every 104 residues. In food allergens, 61% of the cation–π interactions have the energy less than –4 kcal/mol, whereas about 25% of these interactions have similar energy in globular proteins (Gallivan, 1999).

Food allergens have the electrostatic energy more than two times stronger than the van der Waals energy for the interacting pairs containing Lys. The comparison on the strength of cation–π interaction energy of Arg and Lys pair in food allergens reveals that Arg pairs have stronger cation–π interaction energy than that with Lys. The average cation–π interaction energy of Arg and Lys pairs in food allergens is –4.97 and –4.00 kcal/mole respectively. This result indicates that the cation–π interaction play an important role in the stability of food allergens.

The contribution of positively charged residue, Arg is higher (18.92%) than that of Lys (5.47%) in food allergens to form cation–π interactions; this is similar to the trend observed for globular proteins (Gallivan, 1999). The sequential distance between the cationic and aromatic residues for each of the cation–π interactions revealed that majority of the cation–π interactions in food allergens are influenced by long range interactions as observed in structural proteins. This result reflects the importance of long range interactions for the stability of all classes of proteins (Gromiha, 1997 and Selvaraj, 2003). The conservation score for all cation–π interactions forming residues in food allergens revealed that cation–π interaction forming residues in food allergens are less conserved than that in other structural class of proteins.

The percentage calculation of cation- π interaction forming residues of food allergens at various range of solvent accessibility inferred that all of the aromatic residues, Phe, Tyr and Trp prefer to be in the interior of food allergens. This is similar to membrane proteins wherein most of the cation–π interactions forming aromatic residues are buried (Gromiha et.al., 2005). On the other hand, in DNA binding proteins Tyr prefers to be at the surface and Trp has almost equal preference in all ranges of solvent accessibility (Gromiha *et.al.,* 2004). Cation–π interaction forming residues in different secondary structures of food allergens reveals that none of the cation–π interactions residues of food allergens prefers to be in the secondary structure of turn.

Interestingly, analysis of stabilizing residues in food allergens indicates that only five residues, viz. Arg 43, Lys 93, Tyr 177, Tyr 170, and Phe 83, respectively in thaumatin-like protein in banana (1z3qA), α-lactalbumin in cattle (2g4nE and 2g4nF), Actinidin in kiwi (2actA) and papain in papaya (2cioA) identified as stabilizing residues are also involved in cation–π interactions. This result indicates that the cation–π interactions have distinct roles in the stability of food allergens compared with other conventional non-covalent interactions including hydrophobic, electrostatic, hydrogen bonds, van der Waals etc., as reported for DNA (Gromiha et.al., 2004) and RNA binding proteins (Michael, 2005).

CONCLUSIONS

We have analyzed the influence of cation–π interactions to the stability of food allergen protein structures. We found that 68% of the considered food

allergens exhibit cation–π interactions and the contribution of Arg is higher than Lys to form cation–π interactions. The cation–π interactions are mainly formed by long range interactions and Arg-Trp has the strongest cation–π interaction energy among all residue pairs. Secondary structure and solvent accessibility of the food allergens reveals that cation–π interactions forming cationic residues prefer to be in β-strands and aromatic residues Phe, Tyr and Trp are respectively in β-strands, α-helix and coil regions. While Arg and Lys prefer the exposed environment, the cation–π interaction forming aromatic amino acids prefer to be buried. The cation–π interactions have distinct roles to the stability of food allergens with other conventional non-covalent interactions. Further, the cation–π interaction forming cationic and aromatic residues play an important role in the stability of food allergens. The results obtained in this work will be helpful to understand the contribution of cation–π interactions to the stability and specificity of food allergens.

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