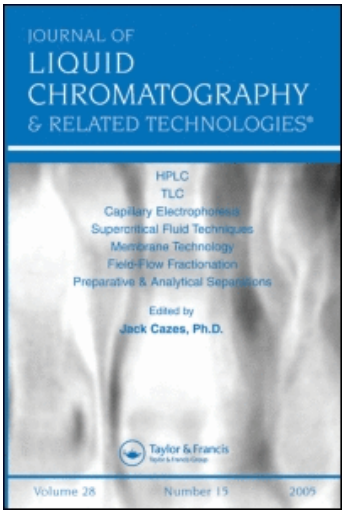

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INFLUENCE OF CATION-π INTERACTIONS IN MESOPHILIC AND THERMOPHILIC PROTEINS

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INFLUENCE OF CATION-π INTERACTIONS IN MESOPHILIC AND THERMOPHILIC PROTEINS

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ABSTRACT

Understanding the factors responsible for exhibiting extreme stability of thermophilic proteins is a challenging task. In this work, we analyze the influence of cation-π interactions to enhance the stability from mesophilic to thermophilic proteins. We found that thermophiles contain higher occurrence of Arg and less occurrence of Lys than mesophiles, which indicates that the replacement of Lys to Arg enhances the stability. In thermophilic proteins, more numbers of Trp would experience cation-π interactions than mesophilic proteins, while the contribution of Tyr is significant in mesophilic proteins. The side chain of Arg is more likely to be in a cation-π interaction of mesophiles than thermophiles. The cation-π interaction energy is significantly different between mesophilic and thermophilic proteins belonging to the same family.

Further, the cation-π interaction energy exhibited by the pair of amino acid residues, Arg–Phe, Lys–Phe, and Lys–Tyr is more effective in thermophiles than mesophiles. The results obtained in the present study would be helpful to understand the stability of thermophilic proteins.

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Key Words: Cation-π interactions; Thermal stability; Electrostatic; Van der Waals

INTRODUCTION

Elucidating the factors responsible for exhibiting extreme thermal stability of thermophilic proteins is very important to understand the mechanism of protein stability, as well as to design stable proteins. Consequently, several investigations have been carried out to reveal the role of non-covalent interactions to protein thermostability and the results have been reviewed.[1,2] Recent studies showed that the increase in negative Gibbs free energy change of hydration for native proteins ($-G_{hN}$) increases the stability of thermophiles[3] and the stability may be achieved by a balance between better packing and solubility.[4] Kumar and Nussinov[5] have made an extensive survey about the importance of various factors influencing the stability of thermophilic proteins.

Protein structures are stabilized with various non-covalent interactions, such as hydrophobic, electrostatic, hydrogen bonds, and van der Waals interactions.[6] In addition, the cation-π interaction is recognized as an important non-covalent binding interaction in structural biology.[7,8] Recently, the importance of this interaction is stressed by several investigators in determining the helicity of α-helical peptides,[9] folding of polypeptides[10] etc.

Gallivan and Dougherty[8] surveyed the nature of cation-π interactions in a set of protein structures. Further, the role of cation-π interactions in different folding types of membrane proteins has been reported.[11] However, the influence of cation-π interactions to the stability of thermophilic protein is yet to be explored.

In this work, we analyzed the role of cation-π interactions in both mesophilic and thermophilic proteins. The energetic contribution due to cation-π interactions has been brought out for each protein and for all the six pairs of residues forming such interactions. We observed a significant difference between mesophilic and thermophilic proteins on the role of cation-π interactions, which would be helpful to understand the enhanced stability of thermophilic proteins.

EXPERIMENTAL

Database

A database of mesophilic and thermophilic proteins was set up from 16 different families that were used in our earlier work to explore the important inter-residue contacts for the stability of thermophilic proteins.[12] The Protein Data Bank (PDB) codes for the mesophilic proteins are: 4MDH, 1CDG, 4GPD, 6LDH,

1NPC, 2RN2, 1ST3, 1FCA, 3SDP, 2PFK, 3PGK, 1YPI, 1RDG, 1INO, 2EXO, and 1LPF, and the PDB codes for their respective thermophilic proteins are: 1BMD, 1CIU, 1HDG, 1LDN, 1LNF, 1RIL, 1THM, 1IQZ, 3MDS, 3PFK, 1PHP, 1BTM, 1CAA, 2PRD, 1XYZ, and 1EBD. The three-dimensional structures of all these proteins have been taken from the Protein Data Bank.[13]

Computation of Amino Acid Composition

The amino acid composition for each amino acid residue that was involved in cation-π interactions was computed using the standard formula,

$$\text{comp}(i) = \frac{n(i)}{N}$$

where, $n(i)$ is the number of amino acids of type i and N is the total number of amino acids in a protein. We have calculated the composition for all the five residues (Lys, Arg, Phe, Trp, and Tyr) that was involved in cation-π interactions in both mesophilic and thermophilic proteins.

Estimation of Cation-π Interactions and Their Energetic Contributions

The number of cation-π interactions in each protein has been calculated using the program, CAPTURE developed by Gallivan and Dougherty.[8] We have computed the energetic contribution of cation-π interactions for each mesophilic and thermophilic protein and for all possible pairs of positively charged-aromatic amino acids. The total cation-π interaction energy ($E_{\text{cat-}\pi}$) has been divided into electrostatic (E_{es}) and van der Waals energy (E_{vdw}) and the results are discussed. The electrostatic and van der Waals energies were obtained from the program CAPTURE, which has implemented a subset of OPLS force field[14] to calculate the energies.

RESULTS AND DISCUSSIONS

Occurrence of Lys, Arg, Phe, Trp, and Tyr in Membrane Protein Structures

The frequency of occurrence of amino acid residues that experience cation-π interactions, are presented displayed in Fig. 1. We found that Arg has a higher frequency of occurrence in thermophilic proteins than mesophilic proteins and an opposite trend was observed for Lys. This implies that the substitution of

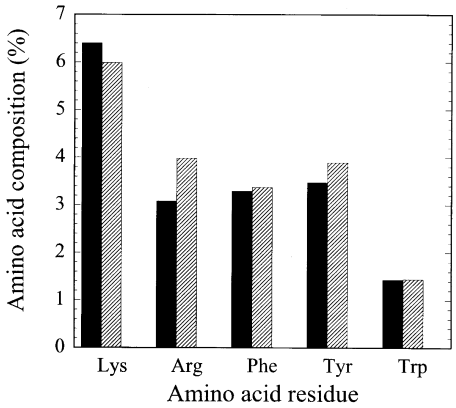

Figure 1. Amino acid composition of aromatic and positively charged residues in mesophilic and thermophilic proteins. filled column: mesophilic proteins; slant column: thermophilic proteins.

Lys into Arg enhances the stability of thermophilic proteins, which agrees well with previous observation.[4,15] Further, the higher occurrence of Tyr in thermophiles suggests that the substitution of aromatic amino acids into Tyr increase the stability. This result was supported by our previous analysis that the replacement of Trp by Tyr enhances the stability.[4]

Relative Contribution of Amino Acids Involved in Cation-π Interactions

The relative contribution of each of the five amino acid residues that are capable of forming cation-π interactions in mesophilic and thermophilic proteins are shown in Fig. 2. We found that more numbers of Trp are involved in cation-π interactions in thermophiles than mesophiles. An opposite trend was observed for Tyr, which experiences more cation-π interactions in mesophiles than thermophiles. Also, Arg is more likely to be in cation-π interactions in mesophilic proteins than thermophilic proteins.

Energetic Contribution of Cation-π Interactions in Mesophilic and Thermophilic Proteins

The number of cation-π interactions in each of the mesophilic and thermophilic proteins and their energetic contribution are presented in Table 1.

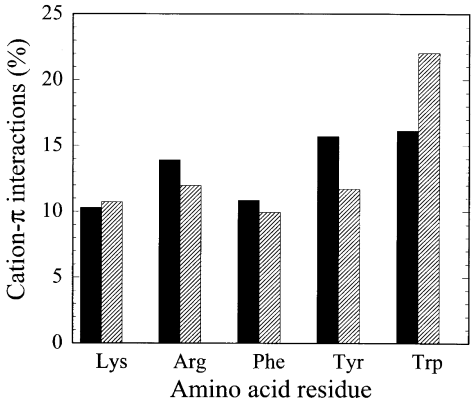

Figure 2. Histogram showing the percentage of aromatic and positively charged residues contributing for cation-π interactions in mesophilic and thermophilic proteins filled column: mesophilic proteins; slant column: thermophilic proteins.

Mesophilic Proteins

The number of cation-π interactions varies for different proteins; it is zero in 1FCA and 13 in 1CDG. Although the chain length is similar in 1NPC (317 residues) and 2EXO (312 residues), the former contains only two, while the latter contains seven cation-π interactions. Further, the strength of cation-π interaction differs in protein structures; it is −6.61 kcal/mol and −10.79 kcal/mol, respectively, for 3PGK and 1NPC, both of them contain two cation-π interactions. The decomposition of energies into two components, electrostatic and van der Waals showed that the electrostatic energy is two times stronger than van der Waals energy, similar to globular proteins.[8]

Thermophilic Proteins

In thermophilic proteins, the maximum number of cation-π interactions is found to be 10 for 1CIU, whereas its mesophilic counterpart contains 13 cation-π interactions (Table 1). The proteins, 1HDG and 1RIL experience only one cation-π interaction and the strength of the cation-π interaction energy is −2.67 and −8.45, respectively. The proteins in the ferredoxin family (1FCA and 1IQZ) contain no cation-π interaction and it might be due to less frequency of positively charged and aromatic residues in these proteins. From Table 2, we observed that about 50% of the families of thermophilic proteins

Table 1. Energetic Contribution Due to Cation-π Interactions in Mesophilic and Thermophilic Proteins

PDB Code	$N_{\text{cat-}\pi}$	$-E_{\text{es}}$	$-E_{\text{vdw}}$ (kcal/mol)	$-E_{\text{cat-}\pi}$
Mesophilic proteins				
4MDH	4	18.46	7.55	26.01
1CDG	**13**	42.04	19.46	61.50
4GPD	2	4.58	2.18	6.76
6LDH	3	13.57	4.48	18.05
1NPC	**2**	6.37	4.42	**10.79**
2RN2	1	1.78	2.71	4.49
1ST3	1	1.79	2.19	3.98
1FCA	**0**	0.00	0.00	0.00
3SDP	3	8.25	2.50	10.75
2PFK	3	8.57	6.51	15.08
3PGK	2	4.86	1.75	**6.61**
1YPI	4	15.55	3.78	19.33
1RDG	1	3.71	0.90	4.61
1INO	4	9.95	3.42	13.37
2EXO	7	25.23	14.96	40.19
1LPF	2	5.24	1.89	7.13
Average	3.3	10.62 ± 10.42	4.92 ± 5.07	15.54 ± 15.30
Thermophilic proteins				
1BMD	3	11.45	8.14	19.59
1CIU	**10**	37.30	15.61	52.91
1HDG	1	2.00	0.67	**2.67**
1LDN	4	12.72	4.98	17.70
1LNF	4	12.77	9.49	22.26
1RIL	1	7.13	1.32	**8.45**
1THM	5	16.41	7.50	23.91
1IQZ	0	0.00	0.00	0.00
3MDS	4	11.39	3.92	15.31
3PFK	1	1.35	2.71	4.06
1PHP	1	6.55	1.64	8.19
1BTM	3	7.91	4.25	12.16
1CAA	1	3.37	0.75	4.12
2PRD	1	2.98	0.41	3.39
1XYZ	8	24.43	13.41	37.84
1EBD	3	8.68	5.37	14.05
Average	3.1	10.40 ± 9.25	5.01 ± 4.57	15.41 ± 13.61

Table 2. Average Energetic Contribution for Each Amino Acid Pair Experiencing Cation-π Interactions

	$-E_{es}$		$-E_{vdw}$		$-E_{cat\text{-}\pi}$	
Amino Acid Pair	Meso	Thermo	Meso	Thermo	Meso	Thermo
	(kcal/mol)		(kcal/mol)		(kcal/mol)	
Arg–Phe	2.70	3.06	2.03	2.58	4.73	**5.64**
Arg–Tyr	3.06	2.58	2.72	2.18	5.78	4.76
Arg–Trp	3.19	3.70	2.68	1.55	5.87	5.25
Lys–Phe	3.06	3.55	**0.68**	0.86	3.74	**4.41**
Lys–Tyr	3.23	3.56	**0.95**	0.91	4.18	**4.47**
Lys–Trp	4.67	4.08	1.07	1.36	5.74	5.44

have a higher contribution of cation-π interaction energy than mesophilic proteins.

Electrostatic and van der Waals Energy for Different Pairs of Amino Acid Residues

We have computed the average electrostatic and van der Waals energy for all the six possible pairs of amino acids, Arg–Phe, Arg–Tyr, Arg–Trp, Lys–Phe, Lys–Tyr, and Lys–Trp and the results are given in Table 2. The van der Waals energy is less than -1 kcal/mol for the pairs Lys–Phe and Lys–Tyr in both mesophilic and thermophilic proteins. The thermophiles have stronger cation-π interaction energy for Arg–Phe, Lys–Phe, and Lys–Tyr than mesophiles. The energy of the other three pairs are weaker in thermophiles than mesophiles. The average cation-π interaction energy is between -4 to -6 kcal/mol in thermophiles, whereas it is between -3 and -6 kcal/mol in mesophiles.

CONCLUSIONS

We have revealed the contribution of cation-π interactions in mesophilic and thermophilic proteins. We found that more numbers of Trp in thermophilic proteins would experience cation-π interactions than mesophiles. Conversely, the residue Tyr in mesophilic proteins is more likely to be in cation-π interactions than thermophilic proteins. There is a significant difference of cation-π interaction energy between mesophiles and thermophiles belonging to the same family. Further, the pair-wise cation-π interaction energy for Arg–Phe, Lys–Phe,

and Lys–Tyr is stronger in thermophiles than mesophiles. The results obtained in the present study would be very useful to understand the enhanced stability of thermophilic proteins.

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