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A COMPUTATIONAL STUDY ON WEAK INTERACTIONS IN HALOALKANE DEHALOGENASE OF MYCOBACTERIUM TUBERCULOSIS H37Rv

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ABSTRACT

Tuberculosis, an epidemic disease, affects one third of world population. The causative agent *Mycobacterium tuberculosis* is targeted in various approaches for the control of tuberculosis. The major factor of bacterial survival in the host depends on the proteins expressed in macrophages to overcome the host immunity. In this study, we carried out computational analysis on weak interactions to study the structural stability of the key enzyme haloalkane dehalogenase of *Mycobacterium tuberculosis* H37Rv (PDB code: 2O2H) expressed during phagocytosis. Cation–pi interactions are one of the critical weak interactions in protein involved in stability of protein. Our results showed that the number of interactions formed by arginine is higher than that by lysine in the cationic group, while those formed by phenylalanine and tyrosine are comparatively higher than by tryptophan in the pi group and that the cation-pi interaction may have a role in protein stability and environmental preference of *Mycobacterium tuberculosis* enzyme haloalkane dehalogenase.

KEYWORDS: Tuberculosis, *Mycobacterium tuberculosis*, Haloalkane dehalogenase, Cation-pi interactions.

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INTRODUCTION

About one third of the world population is infected with *Mycobacterium tuberculosis*, the causative organism of tuberculosis, a disease that triggers humoral response in the body[1]. It is vital to understand the *M. tuberculosis*-host interaction and how the bacteria avoid host defenses and cause disease. Studies have shown that *M. tuberculosis* genes are potentially involved in virulence. Some of these genes and the proteins they encode, as well as newly discovered ones, should provide new bacterial targets that can be used for creating vaccines and drugs[2] as well as more selective diagnostic reagents[3]. Results obtained from genetic and gene expression studies were used for studying the genetics of *M. tuberculosis.* These studies have identified many genes that are significant for virulence or physiological property which are differentially expressed under several conditions, including infection of macrophages or animal models. Haloalkane dehalogenases are microbial enzymes that catalyze the hydrolytic conversion of a chloroalkane or a bromoalkane to the corresponding alcohol and hydrogen halide. The haloalkane dehalogenases belong to the α/β-hydrolase fold family, and cleavage of the carbon–halogen bond proceeds via a covalent alkyl enzyme intermediate. Haloalkane dehalogenases have drawn considerable attention because they catalyze a reaction of great environmental significance - the conversion of an alkyl halide functionality to an alcohol group[3]. The enzyme haloalkane dehalogenases play an important role in catalyzing hydrolytic cleavage of carbon-halogen bonds in halogenated aliphatic compounds and leading to the formation of primary alcohols, halide ions, and protons[4]. Dehalogenase genes, dmbA and dmbB, are thought to be widely distributed among species of *M. tuberculosis*[5]. After phagocytosis, the enzyme haloalkane dehalogenase was found to be highly expressed in K-strain of *Mycobacterium tuberculosis*[6]. The exact function of the enzyme has not been found yet. The crystal structure of *Mycobacterium tuberculosis* haloalkane dehalogenase was elucidated to annotate the function. The weak interaction involved in protein was important in protein stability, mainly cation-pi interactions which was recently found to be a major factor in protein intermolecular interactions[7]. The computational study on weak interactions based on cation-pi interactions, stabilization centres, conservation score and solvent accessibility pattern are valuable tools to confirm the weak interactions involved in protein-molecule interaction. In our present study, we have focused on identifying the weak interactions in *Mycobacterium tuberculosis* haloalkane dehalogenase to characterize the protein stability that could explain its function, if any, in tuberculosis.

MATERIALS AND METHODS

The three dimensional structure of *Mycobacterium tuberculosis* haloalkane dehalogenase was downloaded from PDB[8] (PDB code: 2O2H) and Chain A was selected. The polar hydrogens were added to the protein for interaction study.

Cation- pi interaction

The cation-pi interactions for the query were computed by the program CAPTURE[9], a realistic electrostatics approach method. The program calculates the distance between atom (NZ) in LYS or the atom (CZ) in ARG and the centers of all aromatic rings, phenol, indole, and benzene in residues like TYR, TRP and PHE, respectively. The distance was implemented to form a subset of OPLS force field to estimate the energetic contribution of cation–pi interactions.

Stabilization Centers

The long range contacts of query were studied using predicting stabilization centers. It includes residues involved in cooperative long-range contacts and considered to play an important role in ensuring stability of the protein 3D

structure and preventing unfolding[10]. The server Scide was used for the stabilization center in cation-pi interaction (Table 1).

Computations of Conservation Score

The identification of conserved residues involved in protein sequence favors the cation-pi interactions and were identified by ConSurf server[11]. The parameter conservation score was used for the prediction. The empirical Bayesian algorithm was used for calculating the conservation score for each amino acid residue.

Computations of Solvent Accessibility Pattern

Solvent accessibility pattern for the residues involved in cation-pi interaction was analyzed by NETASA program. The pattern was categorized into buried, partially buried and exposed based on ranges of accessible surface area.

RESULTS

From our computational study, the results showed the weak interactions involved in the query protein haloalkane dehalogenase. The cation-pi interactions analyzed by CAPTURE showed ARG-PHE and ARG-TYR interactions with significant energy (Table 2). Other interactions like ARG-TRP, LYS-PHE, LYS-TRP and LYS-TYR were absent. The overall cation-pi interaction from H-bond analysis tool (HBAT) showed that the intermolecular H bond was more in NO and NN atoms (Fig 1). This confirmed the role of ARG in cation-pi interaction of haloalkane dehalogenase. The interactions of ARG181-PHE4 (Fig 2a) and ARG47-TYR44 (Fig 2b) were visualized in Rasmol and highlighted in red are the residues exposed on surface. Conservation score by ConSurf was calculated to confirm the conserved residues involved in cation-pi interactions. Results showed high score for ARG residues. The solvent accessibility pattern was identified by NETASA and showed that most of the ARG residues and few TRP, TYR residues were exposed on surface and that PHE was buried inside. These results concluded that the weak interactions involved in haloalkane dehalogenase was well studied and critical residues like ARG181, ARG47 may act as active sites for the catalysis reaction carried out by haloalkane dehalogenase. Further studies on haloalkane dehalogenase with inhibitors may help to understand the role of residues which are identified based on cation-pi interactions.

Table 1
Stabilization centre results obtained from Scide

SR	Residue	Cons score	H_P	LRO	SC
1	HIS37	9	21.1	0.0272	1
2	LEU59	8	23.2	0.0306	1
3	VAL106	7	21.9	0.0238	1
4	ALA130	7	28.9	0.0306	1
5	LEU206	7	21.6	0.0204	1
6	LEU240	8	24.2	0.0306	1
7	ALA244	8	22.6	0.0306	1
8	GLY271	7	23.5	0.0238	1

Table 2
Cation-pi interaction results obtained from CAPTURE

Cation	Amino acid	Chain	Pi	Amino acid	Chain	E(es) (Kcal/mol)	E(vdw) (Kcal/mol)	Cation–pi energy (Kcal/mol)
ARG	87	A	PHE	91	A	-1.79	-1.14	-2.93
ARG	181	A	PHE	4	A	-4.76	-4	-8.76
ARG	47	A	TYR	44	A	-4.40	-3.49	-7.89
ARG	229	A	TYR	257	A	-1.13	-1.13	-2.26

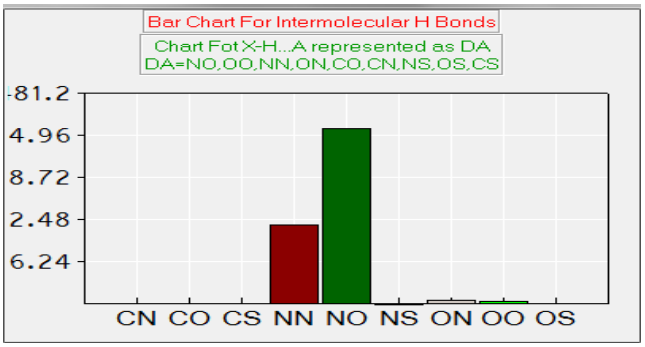

Figure 1
Bar diagram of intermolecular H bonds in haloalkane dehalogenase obtained from HBAT tool.

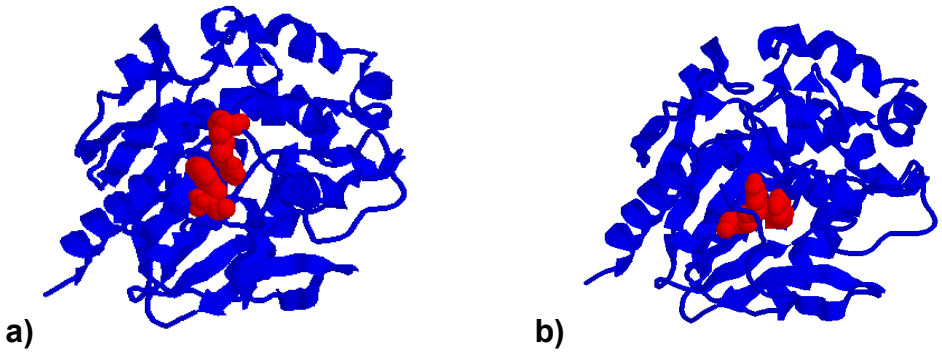

Figure2
a) Cation- pi interaction between ARG181-PHE4 visualized in Rasmol.
b) Cation- pi interaction between ARG47-TYR44 visualized in Rasmol.

CONCLUSION

Our results showed that the number of interactions formed by arginine is higher than lysine in the cationic group, while phenylalanine and tyrosine is comparatively higher than tryptophan in the pi group. Intermolecular H bonds in protein haloalkane dehalogenase were observed with high percentage of NO and NN bonds. The long range contacts are analyzed by stabilizing centres in cation-pi residues includes HIS37 and LEU59 with high confidence score. The conservation score identify the conserved residues of cation in haloalkane dehalogenase and found residue ARG was more conserved in cation-pi interactions residues. Solvent accessibility of the cation-pi residues showed the patterns of ARG, TRP and TYR were exposed to solvent and PHE buried inside. Our results showed the cation-pi interaction may have a role in protein stability and environmental preference of *Mycobacterium tuberculosis* enzyme haloalkane dehalogenase.

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