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Analysis of chloride ion-proline interaction of dehalogenase enzymes by molecular dynamics simulation

Raghunath Satpathy*, Rasmiranjan Behera, Biswajit Nayak
Department of Biotechnology, MIRC Laboratory, MITS Engineering College, Rayagada, Odisha, (INDIA)
E-mail: rnsatpathy@gmail.com

ABSTRACT

This work reports the preference of chloride ion -Proline amino acid interaction among the dehalogenase enzyme. We retrieved 22 dehalogenase and non dehalogenase enzyme available crystallographic structure (from PDB) that contains chloride ion in the structure. Further 3.5 angstrom proximity amino acid residues from the chloride ion was computed for all the selected proteins by an in house built software. The result indicates, in case of the dehalogenase enzymes contains proline residues proximity to chloride ion was uniquely identified. Again a case study was performed to identify the dynamics of the proline residue with respect to the Cl^- ion, by considering the protein having PDB ID 1EDB. The dynamics study during 5 nanosecond MD simulation by GROMACS 4.0.5 reveals that both 223 proline and 226 Val interact with the Cl^- ion more frequently and PRO CA- CL atomic distance is little fluctuated (fluctuation range within 0.5 angstrom). Then to check the structure, function relationship, 3 different substitution mutations were performed on Proline position according to the comparable molecular weight. Then the mutant structures were subjected to 1 nano second molecular dynamics simulation. The result indicates Proline present proximity to chloride ions is important for the dehalogenase enzyme activity.

KEYWORDS

Haloalkane dehalogenase;
Molecular dynamics simulation;
Amino acid distances;
Chloride ion

INTRODUCTION

Beginning from millions of years ago, the evolution has optimized protein structures to fulfil many efficient functions such as enzyme, molecular motor, transport protein, receptor proteins etc. But currently an unresolved query rises about the functional constraints exactly are imposed on a protein structure. Sequence and structure conservation patterns provide little valuable hints in this regard[1-2-3]. Nevertheless, such data is typically local and limited to a specific class of proteins. Yet the recent study demonstrates, the biochemical function of a protein is commonly due to the proper topology of the polypeptide chain[4]. Also the placement of *key amino acids* in the three-dimensional space along with the nature of interacting cofactors[5]. Many types of cofactors often determine the folding attributes of pro-

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teins and therefore, one of the significant steps is to identify the cofactor and its interaction with the protein[6-7]. The proteins bind to varied types of cofactors such as ATP, NADP, halides, pyridine, cyanate, and so forth[8]. The most widely found ions that display diverse functions in many hydrolase families of proteins are halide ions (anions). Binding of amino acids with this halide ions is known to have an effect on the overall protein conformation[9]. in addition to this the chloride ion plays a wide range of roles such as stabilizing the active conformation of a protein, may have role in playing a structural role or acting as catalysts in several catalytic and regulatory processes[10]. Moreover, in many cases ions are critical for crystal formation as the ions mediate crystal contacts between proteins has been studied[11]. Studies describing the geometry of chloride ion binding sites within proteins and in several molecule structures were recently extensively discussed[12].

Our objective is to analyze the properties of chloride ion binding sites in some selected hydrolase groups of enzyme. In particular, we have studied the effect of chloride ion interaction with the proteins by using certain computational tool.

EXPERIMENTAL

Data set under investigation

This work is based on the protein structures available in the PDB database. All structures in PDB which contain at least one chloride ions are included, and having a single chain by advance searches 2 data sets were selected, one for dehalogenase and other for dehalogenase.

Calculation of amino acid interacting from the chloride ion up to 3.5 angstrom

We used ion distance calculation by an in-house based software. It takes the user given ion about the specific ions, chains, user-defined distance range in angstrom etc. and computes the amino acids from the also includes the crystallographic water residues. The computation ocuurs by calculating simple the Cartesian coordinates given in the PDB.

Distance = ((x[1]-y[1])2 + (x[2]-y[2])**2 + (x[3]-y[3])**2)**0.5**

Whereas x [1], x [2], x [3] are x, y and z coordinates of the user defined amino acid/ion and y [1], y [2], y [3] are other amino acid coordinates present in PDB. The amino acid that are within the range of 3.5 angstrom were observed. So it is possible to compute the neighbour amino acids about the given ion in an protein.

MOLECULAR DYNAMICS STUDY AND MUTANT GENERATION

1EDB.pdb ID was selected from the data set and molecular dynamics simulation was done to study more about the ion-amino acid interaction as a case study. MD simulation is one of the popular computational methods that calculates the time dependent behavior of protein and provides detailed information in terms of fluctuations and conformational changes. For MD simulation, GROMACS 4.0.5 (Groningen Machine for Advanced Chemical Simulations) software was utilized (*www.gromacs.org/*). It is a freely available and open source molecular dynamics simulation software mainly designed for simulations of biochemical molecules like proteins, lipids and nucleic acids. The force field GROMOS96 43a1 was used for the present simulation purpose. The protocol for simulation includes placing the initial protein structure in a cubic box filled with water, followed by energy minimization of the system without restraint using the steepest descent method for maximum up to 300 steps. The molecular dynamics simulation was set at temperature 300 K. The P-LINCS algorithm was used with a dielectric constant of 1 and a time step of 2 Femto second (fs) was used for MD simulation purpose with pressure kept constant during the MD simulations.

MUTANT GENERATION AND ANALYSIS

From the MD simulation analysis most frequently amino acids were identified that interacts with the chloride ion and then various mutants were generated. Further, all the mutants were again subjected to an MD simulation at 1 ns to check their suitability, residue wise fluctuation and the effect of proline

TABLE 1 : Showing the selected PDB ID considered for the present computational study

Group	PDB ID	REMARK
Group I	1EDB,1G4H,1G42,1IZ7,1K63,1PWZ,1QQ6,1QQ7,2BFN,2NO5,2O2H,3G9X,3R3U,3R3V, 3R40,4DCC,4E46, 4F5Z,4F71,4IXT,4IXW,4IY1	Dehalogenase
Group II	1HJO,2FOM,2FYQ,2G4W,2OKB,2RB4,2VYO,2WTA,2YD3,2YV5,2Z2K,3D44,3F9O,3MZQ,3RDR,3UWB, 3ZVL,4ABE,4AHN,4GEN,4I69,4J1A	Non-dehalogenases (Other hydrolases)

substitution at that location.

RESULTS AND DISCUSSIONS

All the structures in the PDB, which possess biologically important chloride ions bond with them, were analyzed for their characteristics of interaction with amino acids with close proximity to 3.5 angstrom. As mentioned in the Materials and Methods section, all total 44 PDB were selected contains 22 structures for dehalogenase and 22 for non-dehalogenase TABLE 1. All the structures were analyzed by the in-house build tool for distance calculation. The amino acid preference towards the chloride ion binding is shown in Figure 1 and Figure 2.

In Figure 1 along with water molecule the proline amino acids are more frequent followed by Trp and Asn respectively. However, in case of the non - dehalogenase group, along with water, most frequent amino acids are Asn. All the enzymes selected are hydrolases, hence it rquire water for their catalysis and Asn is common to them, might have involved in catalytic process[13-14]. So the details about kinetic effect on chloride ion – proline interaction should be explored.

The result indicates proline is most frequently required in the event of the dehalogenase data set, that trend does not observe in the non-dehalogenase data set. This suggests the chloride ion having preference for the specific amino acids in different groups of the proteins, might involve in the catalytic (and stability) function. Further study more about this interaction a case study was carried out by performing a 5 ns, MD simulation in water by considering PDB ID 1edb as a case study. After simulation the fluctuation of amino acids was examined with respect to the chloride ions. The fluctuation of previously calculated amino acids like (GLU 56, TRP 125, TRP 175, PRO 223, and VAL 226) were analyzed and depicted in Figure 3. This suggests about

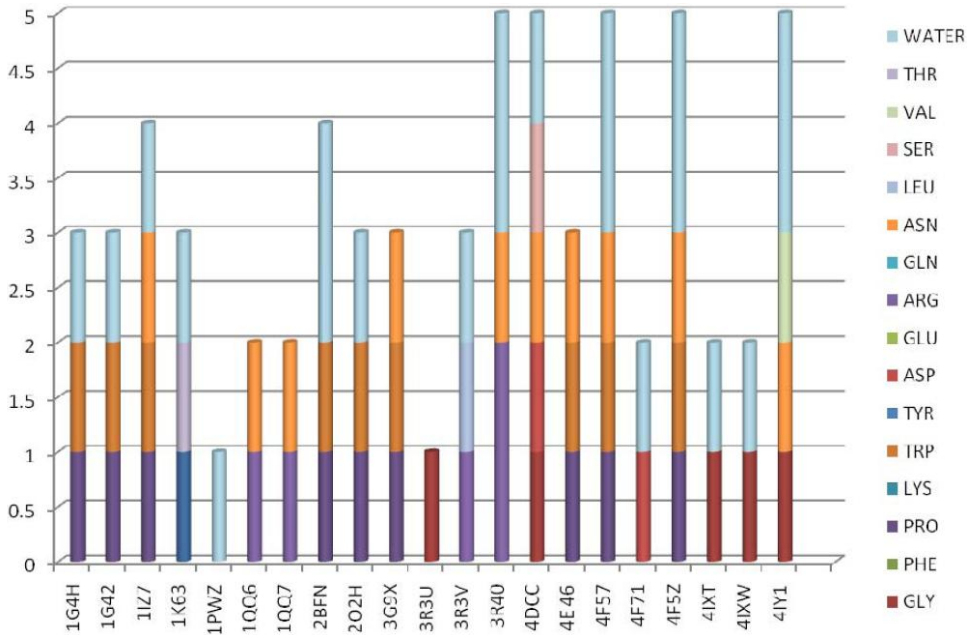

Figure 1 : Showing cchloride interaction amino acids of dehalogenase data set

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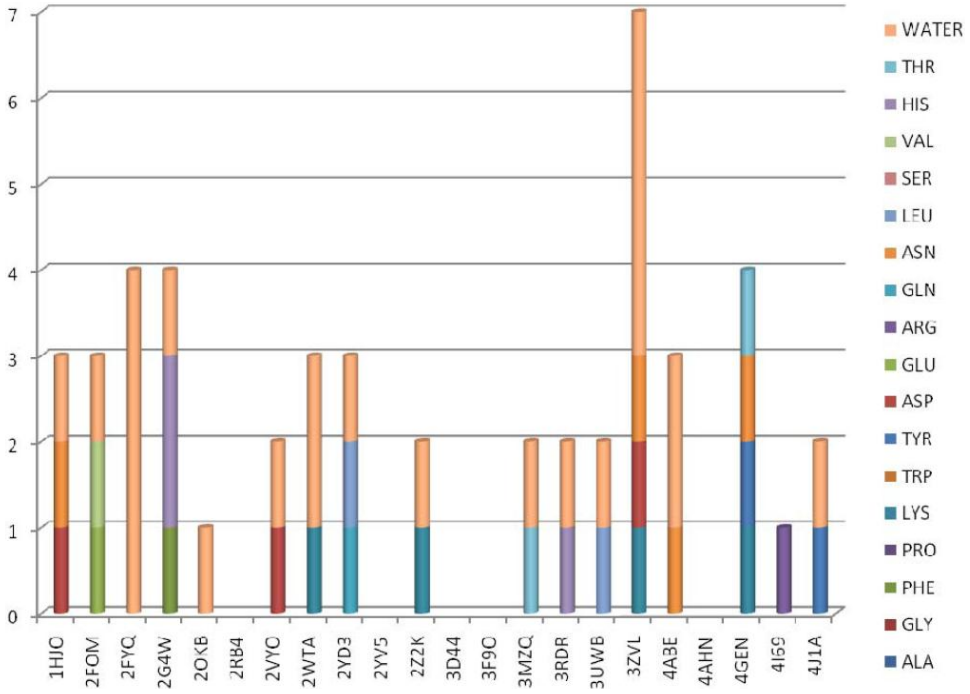

Figure 2 : Showing chloride ion interaction in Non dehalogenase data sets

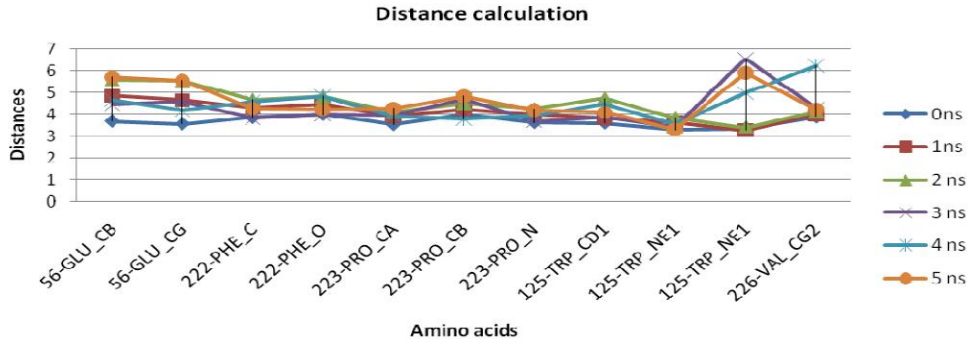

Figure 3 : Chloride-amino acid interaction after 5 ns molecular dynamics simulation in 1edb.pdb protein

the perpetual interaction of the chloride ions with the proline residues. The considered protein (1edb) is a member of haloalkane dehalogenase (from the source *Xanthobacter autotrophicus*) and contains 310 amino acid sequences[15]. Previously hydrolytic mechanism of the enzyme was studied and it indicates about its product as corresponding alcohols and halide ions. Reactions in the active site also requires a series of complicated responses, including an SN2 reaction yielding an alkyl enzyme intermediate and a hydrolysis reaction hydrolyzing the intermediate. This procedure seems to take after an ordered step by releasing the producing halide ion, which is noncovalently bound nearby the catalytic pocket of DhlA[16-17]. Proline amino acids might involve to facilitates the mechanism.

MUTANT GENERATION AND MOLECULAR DYNAMICS STUDY

Further 3 mutants were generated by substituting the 223 position of proline in the protein having PDB ID 1edb. Then 1 nanao second molecular dynamics simulation was performed by Gromacs software.

Three different mutations viz. 223 Proline to Alanine,Valine and Threonone respectively, were performed by pymol v0.99 mutagenesis module (*www.pymol.org). It* is expected that as like to other protein features the anion (chloride ion) presence of a protein may be stabilizing or destabilizing toward protein architecture, depending on types of proteins[17-18-19]. In our analysis, it was obtained that,

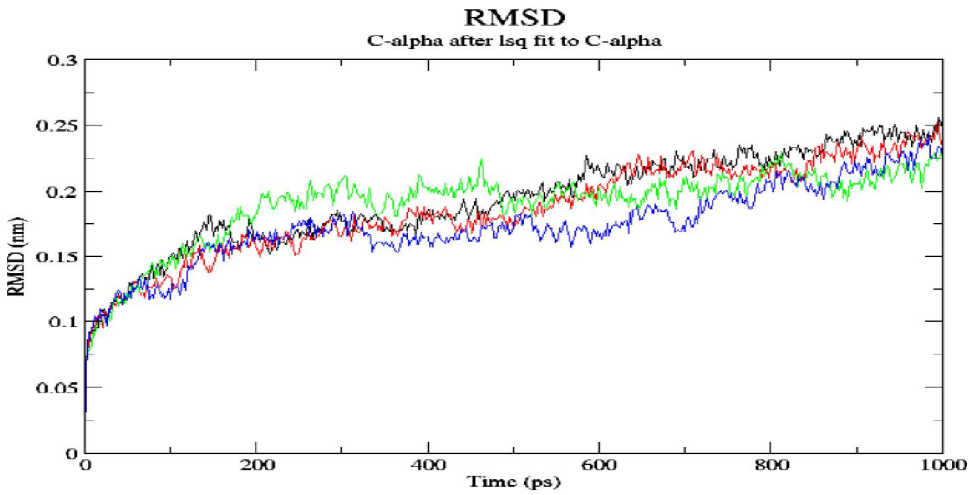

Figure 4 : RMSD profile of the mutant proteins (Black –wild type, red 223P/A, blue 223P/V, and green 223P/T)

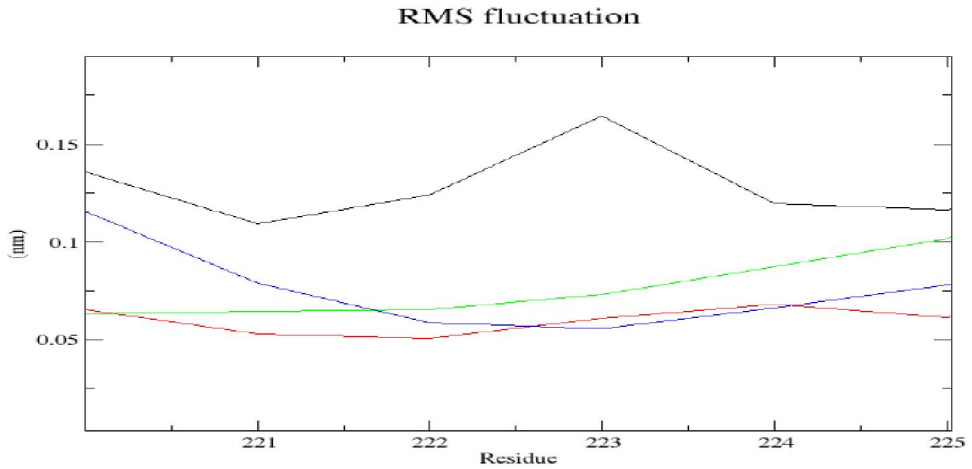

Figure 5 : Residue wise fluctuation of amino acids in the position 223 (Black –wild type, red 223P/A, blue 223P/V, and green 223P/T)

proline amino acid mutation having no such significant effect on the protein structure as noted from the RMSD profile Figure 4. The mutation also show little effect on the fluctuation during 1 ns molecular dynamics simulation Figure 5. Proline itself is an known destabilizing residues (helix breaker), hence mutation having no such effect on stability[20]. Many analysis has been performed for the finding halide binding motif observed in protein crystals are mostly stabilizing in its function in many aspects.[21-22-23-24]. However presence of proline closest to the chloride ion in case of haloalkane dehalogenase might be responsible for the catalytic action. Hence further analysis of the geometry of chloride ion as well as the effect of physical environment analysis of the amino acid interaction during catalytic process is essential.

CONCLUSION

In this work, we suggest it is essential to study about the presence of chloride ion especially in the hydrolases. We also present an in-silico analytical method of the proline amino acids and chloride ion involved in (ion-protein interactions) in case of haloalakne dehalogenase. The analysis shows positive preferences of chloride ion towards particularly Proline amino acids in the case of dehalogenase group of enzymes. Also it has been seen by mutating the proline residue having negiligibe effect on protein stability. Our approach may be employed for deducing the role of chloride ion interaction with proline in the catalytic reactions haloalkane dehalogenase enzymes that has not been identified

till today.

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