Chapter 4

AN INDIAN BUTYRYLCHOLINESTERASE VARIANT L307P IS NOT STRUCTURALLY STABLE: A MOLECULAR DYNAMICS SIMULATION STUDY
4.1. ABSTRACT

The hBChE activity is less than 1% in the serum of silent variant individuals of Vysya community in India. Such individuals are homozygous for a point mutation at codon 307 (CTT→CCT) resulting in the substitution of leucine 307 by proline which make the protein to disappear from the serum. The reason for the disappearance is not because of the defective transcription mechanism as elucidated in the previous chapter. The other possible reason has also not been explicated till date. Based on this background, we performed molecular dynamics simulation to probe the structural stability of this BChE variant (L307P) in comparison with wild and also with other BChE variants (D70G, E497V, V142M) having differential esterase activity. The simulation of all the mutants except D70G showed a much larger Cα root mean square deviation from the wild BChE crystal structure, implicating the overall conformational disturbance. Further analysis revealed that secondary structure of such mutant proteins was also not stable. The orientation of the catalytic triad is also distorted in all the mutants. The distance between δ nitrogen of His438 to ε oxygen of Glu325 and ε nitrogen of His438 to γ oxygen of Ser198 were highly altered in L307P mutant than the wild and other three variants throughout the simulation. Such disparity of distances between the catalytic residues may be due to the change in the protein conformation attributing to their differential catalytic activity. Our studies thus prove that the Indian BChE L307P mutant with negligible activity is possibly due to its structural instability when compared to other BChE variants.
4.2. INTRODUCTION

The genetic variants of hBChE are of clinical interest as this enzyme is involved in several pathophysiological conditions in humans. BChE serves as a biomarker of accidental exposure to OP and carbamate pesticides. As well, the enzyme inactivates the local anesthetics, muscle relaxants and analgesics. For several years, the research focus of BChE was in the field of anesthesia due to the inability of some individuals to clear the muscle relaxant SuCh from their system that was administrated during surgery, resulting in prolonged life-threatening apnea. BChE was found to be the principal enzyme involved in the termination of the action of SuCh and individuals who experienced prolonged apnea had BChE variants with reduced esterase or no esterase activity [Neşe Çokuğraş, 2003].

Sixty eight variants of BCHE gene have been identified and these mutations invariably result in decreased levels of esterase activity. In the A variant, (GAT→GGT; D70G) the aspartic residue at codon 70 is replaced by glycine with 30% lower esterase activity than normal [McGuire et al., 1989]. Another variant, the J variant results from a mutation at codon 497 that causes a change in glutamic acid residue to valine (GAA→GTA; E497V) leading to 66% lower esterase activity relative to BChE-wild type [Bartels et al., 1992]. In the H variant, the valine residue at codon 142 is replaced by methionine (GTG→ATG; V142M), resulting in a protein that has 90% lower esterase activity than the wild-type enzyme [Jensen et al., 1992]. Thus lower esterase activities observed in humans are invariably the result of mutations in BCHE gene which are inherited.

In the Indian population, in a particular community named Vysya, we identified a new BChE variant L307P. About 4% of this population is homozygous for the variant at codon 307 (CTT→CCT), resulting in substitution of leucine 307 by proline. Such
individuals have only 1% esterase activity when compared to normal, and are considered as silent variants [Manoharan et al., 2006]. Even though these individuals are leading a normal life [Manoharan et al., 2007, Manoharan et al., 2007], the BChE protein is totally absent in their serum and the reason for the disappearance of the protein in the serum has not explicated till date. The genotypic screening of the community showed (chapter 2) that 96% of the population are the carriers of the mutant allele.

Instability of proteins can be elucidated by molecular dynamics (MD) simulation, which enlightens the structural conformation of the protein. We carried out MD simulation study to understand the structural conformation of L307P variant of BChE protein. In order to comprehend structure/function relation, simulation parameters of L307P mutant were correlated with wild and other known mutants of BChE, which exhibit different levels of esterase activity.

4.3. MATERIALS AND METHODS

The crystal structure of hBChE (PDB: 1P0I) was used as the starting point. The missing residues were added manually using Swiss PDB viewer [Guex et al., 1997]. Keeping this structure as the template, the desired mutations were designed. All the MD simulations were performed using GROMACS 4.5 [Berendsen et al., 1995; van der Spoel et al., 2005; Hess et al., 2008] with 53a6 force field [Oostenbrink et al., 2004]. The initial structures were solvated with three-point transferable intermolecular potential (TIP3P) water molecules and chloride ions with an appropriate number into a rectangular box to neutralize the system; the dimension of the box was ensured such that any protein atom was at least 8 Å away from its wall. After energy minimization, MD simulations were performed for 10 ns at constant temperature (300 K) and pressure (1.0 bar) with periodic boundary conditions, particle-mesh Ewald summation
[Darden et al., 1993], and a 4-fs time step to heat and equilibrate the system. The Lincs algorithm [Hess et al., 1997] to constrain bond lengths and the rototranslational constraint algorithm were used. The initial velocities were taken randomly from a Maxwellian distribution at 300 K, and the temperature was held constant by the Berendsen algorithm [Berendsen et al., 1984]. The Lennard-Jones and electrostatic interactions within the short-range cut-off of 1.0 nm were evaluated in every time-step.

The parameters analysed were: root mean square deviations (RMSD) of backbone to quantify the conformational changes between the mutated and wild hBChE, root mean square fluctuations (RMSF) of $C_{\alpha}$ atoms of each amino acid residue, radius of gyration (Rg), which is defined as the mass-weighted positional mean of the distances of atoms from the center of mass to evaluate the compactness and the size of the protein, secondary structure and solvent accessible surface area of the protein. These parameters for wild type and mutants were compared with L307P mutant BChE.

4.4. RESULTS AND DISCUSSION

The Vysya community is a group of people living in southern part of India. Normally, they do not undergo any invasive medical intervention involving the use of anaesthesia. Although the mutated BCHE gene results in the absence of BChE protein in their serum, the in vitro expression of this mutated BCHE gene in Chinese hamster ovary resulted in BChE protein with lower activity [Manoharan et al., 2006]. This finding prompted us to probe the conformational stability of L307P BChE protein. The MD simulation parameters of other mutated versions of BChE protein (D70G, E497V, V142M) showing lower esterase activity were compared along with L307P mutant.
4.4.1. Root Mean Square Deviation (RMSD) / Root Mean Square Fluctuation (RMSF)

To obtain an estimate of the MD trajectory quality and convergence, the RMSD of the backbone from the starting crystal structure (1P0I) was calculated (Fig. 4.6.1). After a rapid increase during the first 5 ns, the protein backbone RMSD average over the next 5 ns of the wild-type trajectory was 2.1 Å. The RMSD values of L307P along with all the other variants were compared to wild type. The mutant D70G which shows about 70% activity is relatively stable as that of the wild protein (Fig. 4.6.1B). The other variants having lower activities showed higher deviation (2.4 – 2.8 Å) from the wild structure (Fig. 4.6.1A, 1C, 1D). The RMSF of the amino acids (I69–S79) of Ω loop that lines the primary entrance of the active gorge of BChE showed only slight fluctuation in all the variants from the wild structure (Fig. 4.6.2). The Ω loop of the protein, which is important for sliding the substrate down to Trp82 residue of choline binding site or cation-π site of the active site, it is not affected by the mutated L307P.

4.4.2. Conformation of the protein

The secondary structure was analyzed as an index of protein conformation by examining the total number of amino acids in all the alpha helixes and beta sheets of the protein. The total number of amino acids in all the alpha helixes (~190 residues) of the variant proteins was drastically decreased by approximately 30 residues when compared to the wild structure (Fig. 4.6.3) but the total number of residues in beta sheets remained almost the same (data not shown). The radius of gyration (Rg) of L307P was found to be similar to that of wild up to 5 ns following a steady increase up to 10 ns of simulation (Fig. 4.6.4A). This indicates that the structure opens up and subject to destabilization. The Rg of D70G was only slightly increased from 5 ns to 8 ns of simulation (Fig. 4.6.4B) indicating its stability and thus accounting for 70% activity. In E497V, the Rg is similar to that of the wild structure (Fig. 4.6.4C). The V142M
variant showed a decreased radius after 6 ns of simulation (Fig. 4.6.4D) indicating the shrinkage of the protein.

4.4.3. Role of catalytic residues

The catalytic triad of BChE protein (Glu325, His438, Ser198) is closely be similar to as that of other serine proteases which is present at the bottom of 20 Å deep active gorge lined by hydrophobic amino acids. The Ser hydroxyl group acts as the nucleophile against the carbonyl group of the substrates while the Glu-His pair is crucial in keeping the Ser OH with nucleophile character. So the distance between $\varepsilon$ oxygen of Glu325 to $\delta$ nitrogen of His438 and $\varepsilon$ nitrogen of His438 to $\gamma$ oxygen of Ser198 of L307P mutant protein was determined during the simulation and compared with other mutants along with the wild protein. In L307P mutation, the distance between His438 and Glu325 was found to be increased after 6 ns of simulation, but the distance between His438 and Ser198 was decreased (Fig. 4.6.5A, 4.6.6A). In D70G, the distance between His438 and Glu325 is increased during the initial 6 ns, whereas the distance between His438 and Ser198 was decreased (Fig. 4.6.5B, 4.6.6B). In E497V, the distances between His438-Glu325 and His438-Ser198 are increased only in the initial time of simulation (Fig. 4.6.5C, 4.6.6C). In V142M, the distances between His438-Glu325 and His438-Ser198 are increased throughout the simulation (Fig. 4.6.5D, 4.6.6D). These fluctuations of the catalytic residues strongly point out that a drastic change in conformation of the catalytic triad of the protein favors alteration in their catalytic activities observed among the variants of BChE. Earlier, an insilico study from our lab has shown that for any serine protease to be catalytic in nature, the bond angle between Ser $O^\gamma$–His $N^{\varepsilon 2}$ in the catalytic triad should lie between $115^\circ$ and $140^\circ$ [Gupta et al., 2010]. Since the distance between Ser and His is altered in the mutants compared to wild protein, the angle between Ser $O^\gamma$–His $N^{\varepsilon 2}$ has also changed, favoring the altered catalytic properties by the mutants.
Molecular simulation studies on BChE protein show that different variants exhibit different conformational changes in its overall structure and the catalytic site thereby changing the affinity of its substrate. Upon changes at the G116 position, severe alterations around the active site region were identified [Vyas et al., 2010]. By comparing the conformational dynamics of BChE, the gating mechanisms have been proposed for their differential substrate specificities. Similarly, simulation of the multimeric form of BChE indicates that two dysfunctional active sites do exist, which have restricted accessibility to substrates [Fang et al., 2011]. By mutational studies, BChE protein was shown to lose the catalytic activity by altered positioning of the substrate. So the loss of catalytic property in proteins like BChE is mainly due to conformational changes pursued by the inability for positioning the substrate for effective catalysis. In all the mutants of our study including L307P, the orientation of the catalytic triad is altered, which makes the substrate unavailable for proper catalysis resulting in proteins with lesser catalytic properties.

4.5. CONCLUSION

Among the four BChE mutants, D70G variant is structurally similar to the wild protein even with the loss of 30% activity. The protein conformation is altered in E497V and V142M variants, which result in distorted catalytic properties. Our results clearly show that in L307P as well as in other mutants, the orientation of the catalytic triad is altered by the discrepancy in the distances between the catalytic residues which could be the reason for different catalytic activities exhibited by different variants. Moreover the higher radius of gyration in L307P mutant shows that the protein opens up, making it unstable and it could be the reason for the disappearance of the protein from the biological system. Our present work is the first study to reveal the protein structural changes in L307P variant of hBChE.
4.6. FIGURES

Figure 4.6.1. RMSD plots of wild and mutant BChE structures. The RMSD of backbone atoms (C, CA, and N) from the starting structure as a function of time for MD simulations of the wild BChE protein compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures.
Figure 4.6.2. RMSF plots of wild and mutant BChE structures. RMS Fluctuation of $C_\alpha$ as a function of residue number of wild BChE protein compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures. The $\Omega$ loop residues (I69-S79), CAS residues (Glu325, His438, Ser198) and the PAS residues (D70, Y332) are slightly fluctuating from the wild structure in all the variants.
Figure 4.6.3. Secondary structure analysis plots of wild and mutant BChE structures. The change in the total number of residues of the alpha helixes throughout the simulation of wild BChE protein was compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures. Approximately 30 residues were decreased in all the mutants confirming the structural disturbances in the secondary structure.
Figure 4.6.4. Radius of gyration (Rg) plots of wild and mutant BChE structures. Rg as a function of simulation time of wild BChE protein was compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures. The highlighted part in L307P mutant shows a high Rg which indicates the opening up of the protein.
Figure 4.6.5. Distance between His438 to Glu325 wild and mutant BChE structures. Distance tracked through MD simulation between δ nitrogen of His438 and ε oxygen of Glu325 of wild BChE protein and compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures.
Figure 4.6.6. Distance between His438 to Ser198 wild and mutant BChE structures. Distance tracked through MD simulation between $\varepsilon$ nitrogen of His438 and $\gamma$ oxygen of Ser198 of wild BChE protein was compared with D70G (A), L307P (B), E497V (C) and V142M (D) variant structures.