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Prediction of Site Directed Mutagenesis of Acetylcholinesterase by Using Hotspot Wizard Tool

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Abstract: ***The present study was predicted mutability position of acetylcholinesterase (PDB ID: 2X8B) by using an online academic tool (HotSpot Wizard, version 3.1). The prediction results were obtained through an output interface in which the functional hotspot of acetylcholinesterase indicated only chain A attached to residues like Glu at 202, Ala at 204 position and Ser at 125 positions, respectively. The pockets were obtained in 1 (catalytic) for all residues in which B - factor values 61.86, 59.91 and 52.35 Å^2, respectively. There was predicted higher mutability rate with the score value of 6 in the same chain A. It is concluded that this predictive study is beneficial to detect antidotes related to the specific protein.***

Keywords: Acetylcholinesterase, Bioinformatics, Protein engineering, HotSpot Wizard, Mutability position

1. Introduction

In vertebrates, cholinesterase (ChE) is an important enzyme found as acetylcholinesterase (AChE; EC 3.1.1.7) in the brain and in neurofibrillary tangles and neuritic plaques. [1, 2] Zhao et al. [3] reported that cholinesterase (ChE) dismisses acetylcholine (ACh) efficiency in synapses.

Generally, cholinesterase inhibitors are called as acetylcholinesterase (AChE) inhibitors or anticholinesterases. These are a group of drugs that block the normal breakdown of acetylcholine (ACh) into acetate and choline, which increased in higher levels and duration of actions of acetylcholine observed in the central and peripheral nervous system. [4]

Cholinesterase inhibitors increased the overall amount of acetylcholine presented. Consequently, the symptoms are observed as overstimulation of the parasympathetic nervous system in which increased level of hypersecretion, hypermotility, bradycardia, miosis, hypotension and diarrhoea may be occurred.

On the other hand, to date the site - directed mutagenesis is a well - known technique for explaining the structural and/or functional role of the amino acid (s) in a biologically active protein. The usage is especially productive when applied to a particular protein of known three - dimensional structure in which the structural evidence permits both coherent planning of a comprehensible repertoire of mutations. [5]

The present study was predicted mutability position of acetylcholinesterase (PDB ID: 2X8B) by using an online academic tool (HotSpot Wizard, version 3.1).

2. Materials and Methods

The crystal structure of protein as acetylcholinesterase, pdb files as PDB ID: 2X8B was selected and incorporated separately in the input interface of HotSpot Wizard (version 3.1) online tool as per the protocols of other investigators. [6 - 13] In this automated prediction study, chains were not specified manually. The predicted mutability position of amino acid as per functional hotspot was studied and exhibited in the results.

3. Results

Table 1 describes the functional hotspot of acetylcholinesterase where only chain A attached to residues like Glu at 202, Ala at 204 position and Ser at 125 positions, respectively. The pockets were obtained in 1 (catalytic) for all residues in which B - factor values 61.86, 59.91 and 52.35 Å^2, respectively.

Table 1: Study of functional hotspots

Studied Protein	Chains	Residues & position	Secondary structures	Pockets & tunnels	Average B - factor (in Å^2)	Mutability rate & score
2X8B	A	Glu & 202	Extended strand (E)	1 (catalytic)	61.86	High & 6
	A	Ala & 204	Alpha helix (H)	1 (catalytic)	59.91	High & 6
	A	Ser & 125	Loop (L)	1 (catalytic)	52.35	High & 9

In Fig 1A - C, it was obtained that the amino acid residues fulfilling the criterion of minimal frequency in the multiple sequence alignment. The wild type varieties were observed Glu (21%), Ala (39%) and Ser (8%) for functional hotspot.

For the mutational landscape, which mainly obtained the estimation of the probability in relation to preservation of protein function for individual substitution at a particular site of Acetylcholinesterase. It was obtained that higher

deleterious mutation in Fig 2B and lower in Fig 2A and Fig 2C.

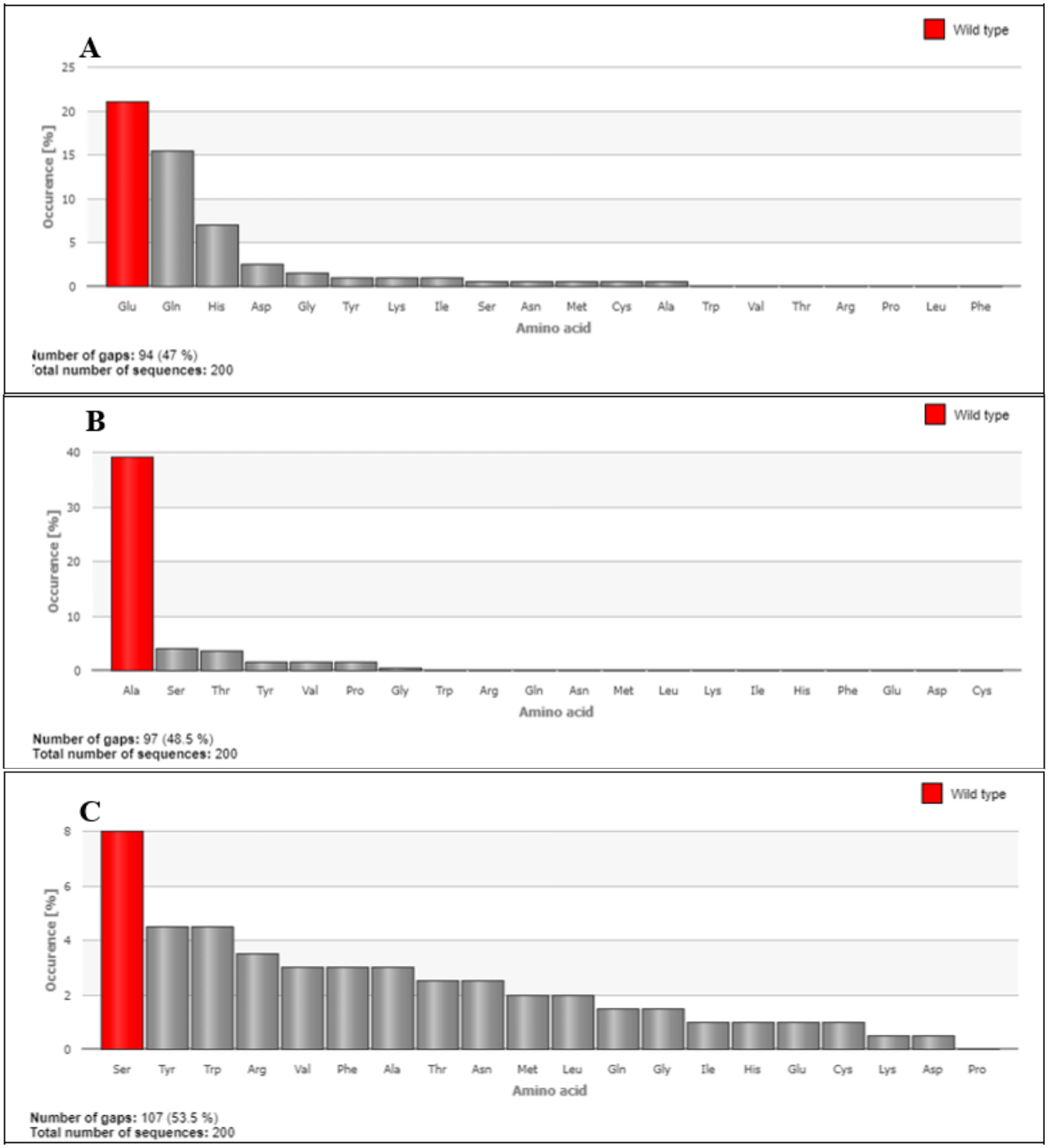
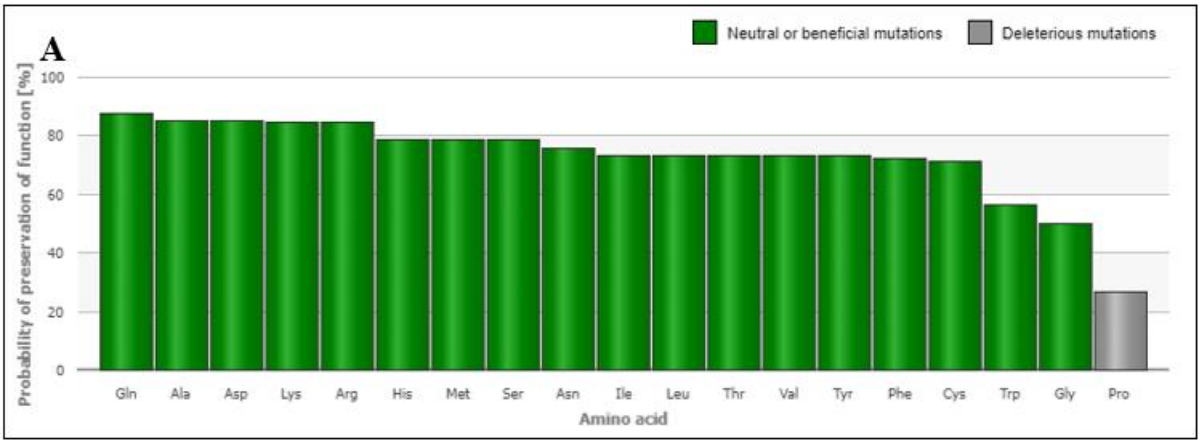


Figure 1 (A – C): Amino acids frequencies as per positions



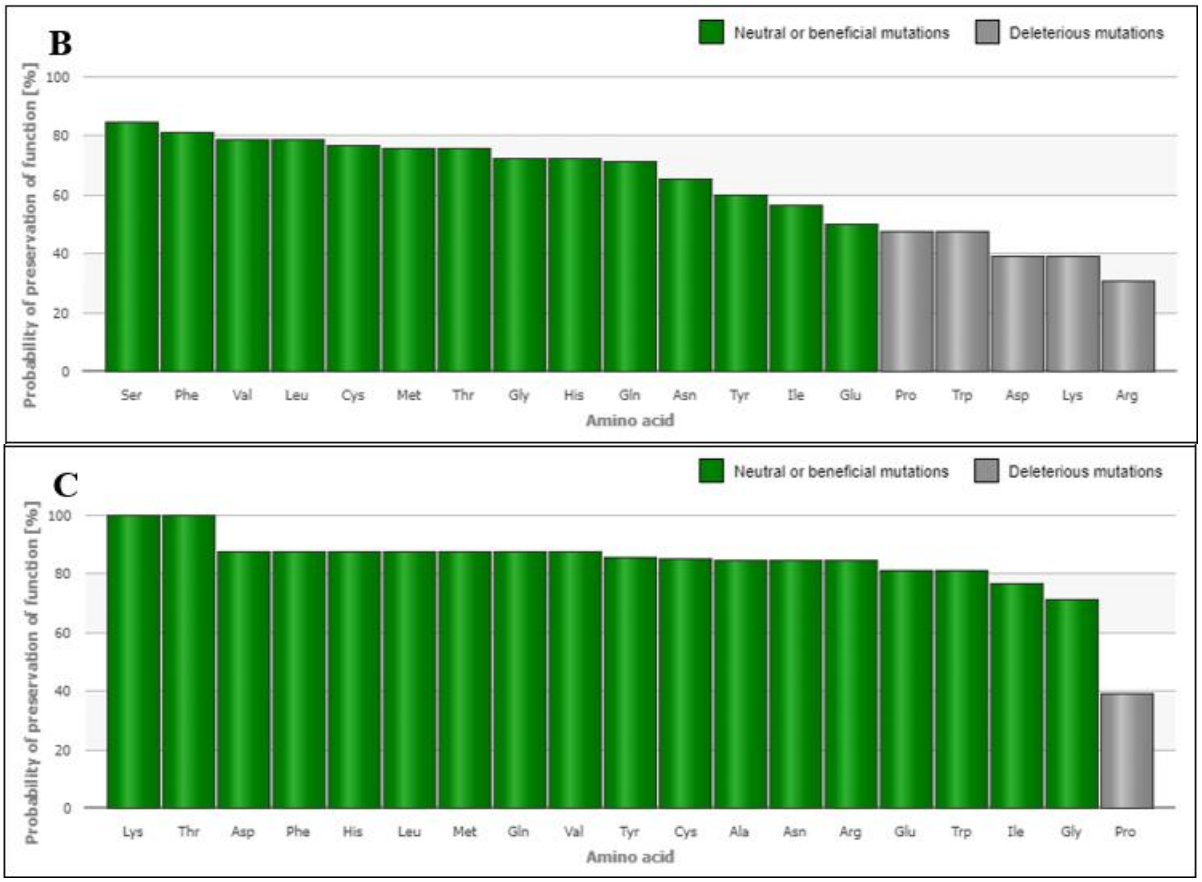


Figure 2 (A – C): Amino acids frequencies as per positions of deleterious mutations

4. Discussion

The prediction data as per functional hot spots, mutability rate and score (higher and 6) were obtained in the specific chain A of the studied protein where only chain A attached to residues like Glu at 202, Ala at 204 position and Ser at 125 positions. The wild type varieties were observed Glu (21%), Ala (39%) and Ser (8%) for functional hotspot. According to Pavelka et al., [6] the ratio of deleterious mutations in the positions assigned as highly mutable by HotSpot Wizard, i. e. with the mutability scores (6 - 9), which were compared in the present study with ratio of deleterious mutations within the whole protein structure. This study helps to know the amino acid conservation in sets of homologous protein to identify likely beneficial as well as deleterious mutations of the studied protein (acetylcholinesterase).

5. Conclusion

In conclusion, HotSpot Wizard (version 3.1) is free online, academic computational tool, which predicted easily the results for site directed mutagenesis of acetylcholinesterase as per several inbuilt databases. This prediction work suggests validating experimental hotspots for this protein related to therapeutic efficacies as antidotes.

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Conflict of interest
Authors declare no conflict of interest.

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