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Porphyrin derivatives as inhibitors for acetylcholinesterase from *Drosophila melanogaster*

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Abstract:
The cure for Alzheimer's disease (AD) is still unknown. According to Cholinergic hypothesis, Alzheimer's disease is caused by the reduced synthesis of the neurotransmitter, Acetylcholine. Regional cerebral blood flow can be increased in patients with Alzheimer's disease by Acetylcholinesterase (AChE) inhibitors. In this regard, Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinatoIron(III) nitrosyl Chloride (FeNOTPPS) were investigated as candidate compounds for inhibition of Acteylcholinesterase of *Drosophila melanogaster* (*Dm*AChE) by use of Molecular Docking. The results show that FeNOTPPS forms the most stable complex with *Dm*AChE.

Keywords: Acetylcholinesterase, Acetylcholinesterase inhibitors, Cholinergic hypothesis, Porphyrin derivatives, Molecular Docking.

Background:
Alzheimer's disease is a costly disease for society. Its causes and progression are not well understood. Current treatments only help with the symptoms of the disease. Alzheimer's disease affects the brain regions of neocortex and hippocampus. The cause for most Alzheimer's cases is still essentially unknown (except for 1% to 5% of cases where genetic differences have been identified). The factors that increase the risk of Alzheimer's disease include age, gender, family history, Down's syndrome, head injury and environmental toxins **[1]**. A large number of potential therapies have emerged for Alzheimer's disease. Among these, some compounds have confirmed effectiveness in delaying the symptoms of Alzheimer's disease. However, the cause and development of Alzheimer's disease is still not well understood. According to Cholinergic hypothesis **[2]**, Alzheimer's disease is caused by decreased synthesis of Acetylcholine. Cholinergic hypothesis proposes that regional cerebral blood flow may be increased in patients with Alzheimer's disease by Acetylcholinesterase (AChE) inhibitors.

Acetylcholine is a neurotransmitter present in many synapses of the nervous system. Acetylcholinesterase (AChE) catalyzes the hydrolysis of Acylcholinesters with specificity for Acetylcholine **[3]**. The reactiontakes place by nucleophilic attack on the carbonyl carbon, acylation of the enzyme and the release of Choline. It is followed by hydrolysis of the acylated enzyme to produce acetic acid, and then re-cycling of the enzymeback to its original state. AChE can also synthesize the neurotransmitter Acetylcholine by the transition of acyl-groups of acetyl CoA **[4–7]**. Acetylcholine is distributed in the cytoplasm of both synaptic endings and synaptic vesicles and it transmits nerve impulse signals in the synapse of myoneural junction.

AChE is bound to cellular membranes of excitable tissues at cholinergic synaptic junctions. Itis also found in red blood cell

membranes. The active enzyme is a monomer with a molecular weight of around 60,000 Daltons. AChE is a α/β protein that contains 537 amino acids. The structure of AChE comprises a 12-stranded mixed β-sheet surrounded by 14 α- helices. The catalytic triad is present in the active-site gorge of the enzyme and it consists of three amino acids, namely Ser238, His440 and Glu367 **[8]**. AChE enzymes have high structural homology. The root-mean-square (RMS) difference between C_α atoms of the vertebrate enzyme (*Tc*AChE) and the insect enzyme (*Dm*AChE) is 0.8 Å **(Figure 1)**. Some regions in surface loops show up to 8 Å difference between the *Dm*AChE and *Tc*AChE structures. The active-site triad of *Dm*AChE(Ser238, His440 and Glu367), the oxyanion hole-forming residues (Gly150, Gly151 and Ala239) and the peripheral anionic binding site (Trp 83) overlap well with *Tc*AChE. The side chains show some differences in conformations from those of*Tc*AChE **[8, 9]**.

Porphyrins are a class of naturally occurring macro cyclic compounds, which play a very important role in the metabolism of living organisms. They have an18-π electron system that makes them aromatic**.** Each Porphyrin molecule contains four pyrrole rings linked via methine bridges **(Figure 2).** The Porphyrin nucleus is a tetradentate ligand in which the space available for a coordinated metal.They have a diameter of approximately 3.7 Å **[10]**. Porphyrin complexes with Mg (II), Cd(II), Zn(II) and Fe(III) can combine with another ligand to form penta-coordinated complexes with square-pyramidal structure **[11]**. In this study, molecular docking was used to predict the strength of binding of Porphyrin-derivatives: TPPS, FeTPPSandFeNOTPPS to *Dm*AChE.

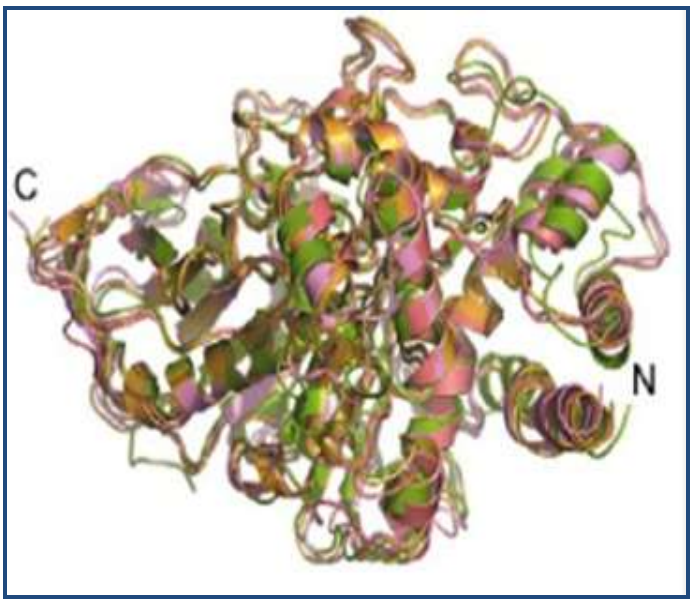

Figure 1: Superimposition of AChE structures from various sources (orange color for *Homo sapiens*, pink color for *Musmusculus*, deep-salmon color for *Torpedo californica*, and split-pea color for *Drosophila melanogaster*) **[10]**.

Methodology:

Ligands

All the three molecules, Tetraphenylporphinesulfonate (TPPS), 5, 10, 15, 20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron (III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis (4-sulfonatophenyl) porphyrinato Iron (III)nitrosyl Chloride (FeNOTPPS), were constructed on a Silicon Graphics Octane2 workstation using IRIX 6.5 operating system. The energies of all the molecules were minimized using theTRIPOS force field and Gasteiger-Hückel charges **[11]** with a convergence gradient of 0.05 kcal/mol/Å. For FeTPPS, the coordinate bonds of Fe(III) and pyrrole nitrogen were defined first before energy minimization. For FeNOTPPS, the coordinate bonds of Fe(III) were first defined with pyrrolenitrogen and then with nitric oxide. The totalenergies of TPPS, FeTPPS and FeNOTPPS after minimization were 67.9, 90.9 and 125.8kcal/mol respectively. The breakup of energies is shown in **Table 1 (see supplementary material).**

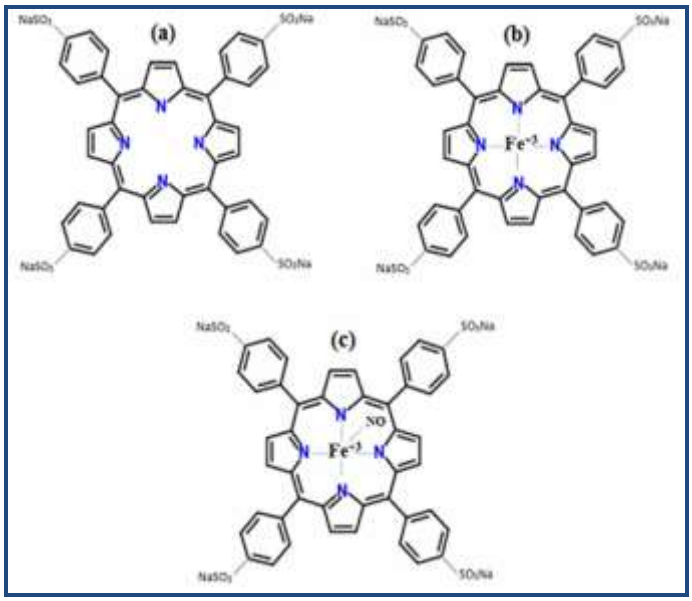

Figure 2: Chemical structures of Tetraphenylporphinesulfonate (TPPS), 5,10,15,20-Tetrakis(4-sulfonatophenyl) porphyrinato Iron(III) Chloride (FeTPPS) and 5,10,15,20-Tetrakis(4 sulfonatophenyl)porphyrinato Iron(III)nitrosyl Chloride (FeNOTPPS) (clockwise from top).

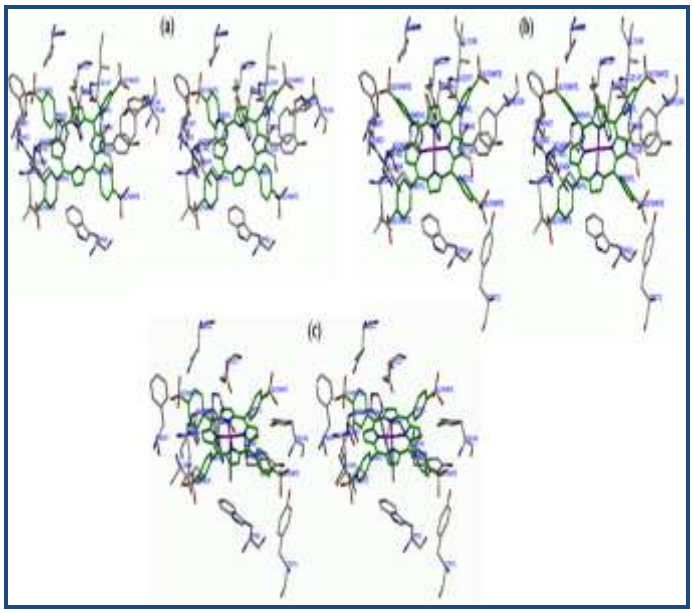

Figure 3: Schematic representation of the sites in AChE. The catalytic triad in the Acyl-binding pocket consists of three amino acids, Ser238, His440 and Glu367.

Molecular Docking

SYBL software was used for docking TPPS, FeTPPS and FeNOTPPS in the crystal structure of *Dm*AChE (PDB code: 1QON). These complexes were then subject to molecular dynamics simulation for duration of 10,000 fs then submitted for energy minimization using a TRIPOS force field and Gasteiger-Hückel charges **[11]** with convergence gradient of 0.05 kcal/mol/Å for TPPS, FeTPPS and FeNOTPPS bound to *Dm*AChE **(Figure 1).**

Analysis of Binding

The strength of binding was determined by use of Scoring Functions. They approximate the free energy of binding of a ligand to a receptor **Table 1 (see supplementary material).** Scoring Functions are expressed as a sum of separate terms that describe the various contributions to binding **[12, 13].** Scoring Functions estimate the binding affinity by taking into account the various terms that can contribute to the binding free energy. These terms may include, for example, van der Waals interactions, hydrogen bonding, de-solvation effects, metal-ligand bonding, etc **[14-17].** A high value of the Scoring Function represents "tight" binding between the protein and the ligand and vice versa.

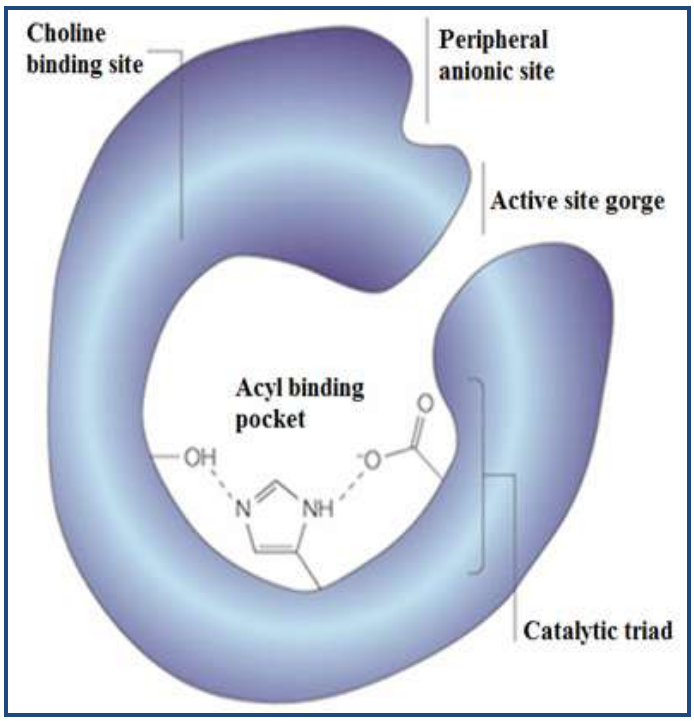

Figure 4: Schematic representation of the sites in AChE. The catalytic triad in the Acyl binding pocket consists of three amino acids, Ser238, His440 and Glu367**.**

Results & Discussion:

An important feature about *Dm*AChE structure is a deep and narrow gorge that is about 20 Å long **(Figure 3).** It penetrates halfway into the enzyme and widens out close to its base. This cavity has been named the "active-site gorge" because it contains the catalytic triad. The active-site gorge of *Dm*AChE is narrower than that of *Torpedo californica* AChE (*Tc*AChE). Its trajectory is shifted by several angstroms. The volume of the lower part of the active-site gorge of *Dm*AChE is half the size of *Tc*AChE. This is due to a shift in the position of the indole ring of Trp83, the replacement of Asp72 in *Tc*AChE by Tyr71, Tyr121 in *Tc*AChE by Met153, and Phe330 in *Tc*AChE by Tyr370 in *Dm*AChE. The active-site gorge of *Dm*AChE is coated with aromatic residues. Their side chains interact with various inhibitors via non-covalent interactions **[18, 19]**. These side chains allow the gorge to accommodate inhibitors by assuming different conformations.

The shape of the acyl-binding pocket at the bottom of the active-site gorge is different in *Dm*AChE versus *Tc*AChE. This is due to differences in two important amino acid residues: Leu328 (Phe288 in *Tc*AChE) and Phe440 (Val400 in *Tc*AChE). These changes change the shape of the acyl pocket **(Figure 3).** One of the differences between vertebrate and insect enzymes is *Dm*AChE's ability to hydrolyze substrates with larger acyl moieties such as butyrylcholine **[20]**. A possible reason for this difference is that residues equivalent to Leu328 and Phe371 of *Tc*AChE in *Dm*AChE are both phenylalanines that form a π–π stacking pair.

Due to the toxic effects of pre-existing AChE inhibitors, current research has been focused on developing new AChE inhibitors or modifying existing ones using computational resources to determine which ligand best fits the AChE binding site. In this study, molecular docking was used to predict the strength of binding of Porphyrin-derivatives: TPPS, FeTPPS and FeNOTPPS with *Dm*AChE. The strength of binding was quantified by use of a Scoring Function that approximates the free energy of binding. **Table 1 (see supplementary material)** gives the different values of the Scoring Function **[15, 16]** obtained by Molecular Docking of TPPS, FeTPPS and FeNOTPPS with *Dm*AChE. The values of the Scoring Function show that FeNOTPPS is energetically the most stable in *Dm*AChE. This can be due to the greater hydrophobicity of FeNOTPPS as compared to TPPS and FeTPPS. The larger size of FeNOTPPS makes it less soluble in water and more stable in the active-site gorge of *Dm*AChE.

Conclusion:

The cure for Alzheimer's disease suggested by Cholinergic Hypothesis involves searching for candidate compounds that can act as inhibitors for Acetylcholinesterase enzyme. The compound, FeNOTPPS, emerged as one such compound from this study. It is energetically more stable than TPPS and FeTPPS when bound to Acetylcholinesterase of *Drosophila melanogaster*. The future direction can be *in vivo* experiments that can check the efficacy of FeNOTPPS for the treatment of Alzheimer's disease.

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Supplementary material:

Table 1: Total Energy and Scoring Functions after Docking of TPPS, FeTPPS and FeNOTPPSin *Dm*AChE

Total Energy after Energy Minimization of TPPS, FeTPPS and FeNOTPPS			
Parameter	**Total energy (kcal/mol)**		
	TPPS	**FeTPPS**	**FeNOTPPS**
Bond Stretching Energy	1.66	1.66	1.75
Angle Bending Energy	65.31	64.25	64.18
Torsional Energy	33.36	33.23	33.66
Out of Plane Bending Energy	0.19	0.19	1.12
1-4 van der Waals Energy	-1.45	-1.72	-1.81
Van der Waals Energy	-15.13	-14.27	-15.85
1-4 Electrostatic Energy	-6.88	-3.02	-2.90
Electrostatic Energy	-9.15	10.57	45.59
Molecule	***Scoring Function Value**		
TPPS	1955738102		
FeTPPS	1604890320		
FeNOTPPS	21918620930		

*Scoring functions are approximate mathematical methods used to predict the strength of the non-covalent interactions (also referred to as binding affinity) between two molecules after they have been docked.