*International Journal of Research Studies in Biosciences (IJRSB)*
*Volume 3, Issue 1, January 2015, PP 37-42*
*ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)*
*www.arcjournals.org*

# Erythrocyte Acetylcholinesterase-Inhibitory Activity of Porphyrin Compounds

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**Abstract:** *Acetylcholinesterase (AChE) inhibition is important because of its health-related implications. Three porphyrin derivatives, 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 tested by Molecular Docking as inhibitors of human Acetylcholinesterase enzyme in erythrocyte membrane ($AChE_H$). These compounds can bind to $AChE_H$ and can result in reversible inhibition. The experimentally observed docking (activity) pattern in terms of stability of binding to $AChE_H$ was found to be: TPPS > FeTPPS > FeNOTPPS. This result demonstrated that binding affinity of these compounds to $AChE_H$ does not increase with the increase in hydrophobicity of these molecules.*

**Keywords:** *Acetylcholinesterase, Acetylcholinesterase inhibitors, Porphyrin derivatives, Molecular docking, erythrocyte membrane.*

## 1. INTRODUCTION

Acetylcholinesterase (AChE) inhibitors are used for the treatment of various disorders such as myasthenia gravis, glaucoma and Alzheimer's disease **[1, 2]**. Knowledge about the structure-activity relationship of AChE is important for understanding catalytic efficiency and drug design. Also, information about the ACh-binding site of AChE can help in understanding the molecular basis for the recognition of ACh by other ACh-binding proteins such as the various ACh receptors. Acetylcholinesterase (AChE) is well-known for its cholinergic functions in the nervous system but this enzyme is also found in other tissues also where its function is still not understood (Fig. 1(a), (b)). AChE is synthesized through alternative splicing as splicing variants, with isoforms including read-through ($AChE_R$), tailed ($AChE_T$) and hydrophobic ($AChE_H$).

The function of Acetylcholinesterase enzyme ($AChE_H$) in human erythrocyte membranes is not completely understood. $AChE_H$ is a glycophosphatidylinositol-linked dimer on plasma membrane. In erythrocyte plasma membranes, different forms of $AChE_H$ enzyme are present as the Yt blood group antigens **[3]**. $AChE_H$ contains a cleavable hydrophobic peptide at the C-terminus with a PI-anchor site. In human erythrocytes, $AChE_H$ is a glycophosphatidylinositol-linked dimer on the surface of the plasma membrane **[4]**. Several studies have suggested that $AChE_H$ is involved in the differentiation of mammalian hematopoietic cells **[5-9]**. $AChE_H$ is present in endoplasmic reticulum and Golgi apparatus of basophilic and polychromatophilic erythroblasts. The process of hemoglobination is reduced when $AChE_H$ is inhibited which suggests a reduction in the rate of erythropoiesis **[10]**. However, some studies have shown that AChE may not play a role in erythroid differentiation. AChE knockout mice ($AChE_\gamma$) displayed normal morphology and normal numbers and types of blood cells, without any abnormalities in hematopoiesis **[11]**.

Electron microscopy of AChE crystals from *Torpedo californica* has provided an insight into the structure of AChE and the changes which occur upon ligand-binding. These studies show that the channel is activated by binding of ACh in pockets located in the extracellular domains of α subunits **[11, 12]**. Ligand-binding rotates the subunits which creates a continuous ion-conducting path through the membrane. A gorge is present near the PAS. This gorge consists of a narrow hydrophobic channel that is about 20 Å long and it connects the PAS site to the active site **[13]**.

The active site of human erythrocyte AChE (Fig. 2) comprises two sites: (i) the peripheral anionic site (PAS), and (ii) the catalytic site. PAS is rich in aromatic amino acids that help in ligand-binding. This hydrophobic region leads ACh to the catalytic site. A catalytic triad is present comprising Glu334, His447 and Ser203. Ser203 is activated by Glu334 and His447. This activation allows the following reaction: the acylation between hydroxyl group of Ser203 and ACh oxygen. A covalent bond between the enzyme and the substrate creates an oxyanion. Here, Tryp86 binds to the trimethylammonium group of Ach **[14]**.

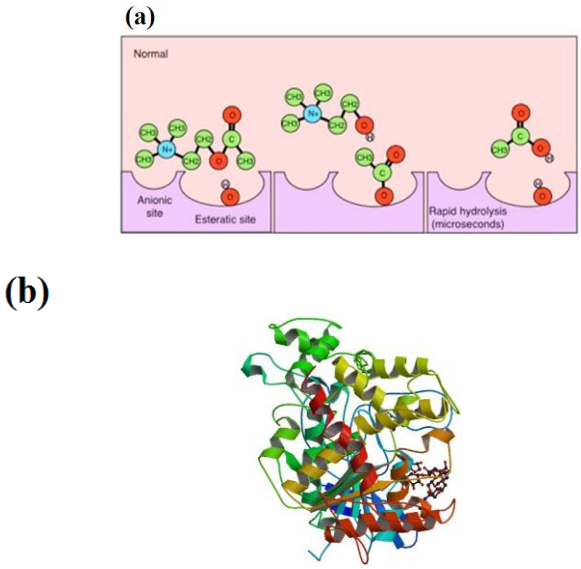


**Figure1.** *(a) Normal Acetylcholinesterase function. (b) Human Acetylcholinesterase (PDB code: 1B41) complexed with Fasculin-II. Protein chains are colored from the N-terminal to the C-terminal using a rainbow (spectral) color gradient.*

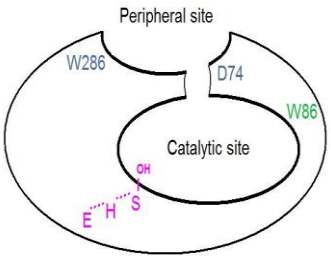


**Figure2.** *Schematic showing the active site of $AChE_H$ consisting of the peripheral anionic site (PAS) and the catalytic site*

The catalytic site, peripheral binding site (PAS) and gorge are all targets for the design of novel AChE inhibitors by use of molecular docking simulations. PAS inhibitors have been shown to inhibit catalysis by sterically blocking ligands from entering and leaving the gorge **[15,16,17,18,19]**. This study used Molecular Docking to test the binding of Porphyrin compounds- 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) to human Acetylcholinesterase enzyme ($AChE_H$) of erythrocyte cell membrane.

## 2. METHODS

### 2.1. Model Building of Ligands

Porphyrins comprise four pyrrole rings connected by methine bridges The models of the three porphyrins used for molecular docking, 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) (Fig. 3), were built by Computer Aided Chemistry (CAChe) software on a Silicon Graphics Octane2 workstation with IRIX 6.5 operating system. The molecular structures were constructed in the "Editor" mode which allows drawing and modification of structures. It creates the atom positions, bond type, configurations, valence, and geometry used as input for all the other CAChe applications. The energies of these molecules were minimized using MM3 calculations **[13]**. For FeTPPS, the coordinate bonds of $Fe^{+3}$and pyrrole nitrogen were defined first before energy minimization. For FeNOTPPS, the coordinate bonds of $Fe^{+3}$were first defined with pyrrole nitrogen and then with nitric oxide. The total energies of TPPS, FeTPPS and FeNOTPPS after minimization are shown in Table 1. The compound, FeTPPS, possesses an energy value which is in between the energies of TPPS and FeNOTPPS. The molecule with the lowest energy was observed to be FeNOTPPS.

### 2.2. Ligand Binding to AChEH

The three molecules, TPPS, FeTPPS and FeNOTPPS (Fig. 3), were docked into the crystal structure of $AChE_H$ (PDB code: 1B41) **[20]** (Fig. 1(a)). The strength of binding was determined by use of Scoring Functions **[21, 22, 23]**. Scoring Functions are expressed as a sum of separate terms that describe the various contributions to binding**.** These approximate the free energy of binding of a ligand to a receptor. The experimentally observed pattern of binding to $AChE_H$ was found to be: TPPS > FeTPPS > FeNOTPPS.

## 3. RESULTS AND DISCUSSION

$AChE_H$ belongs to the $\alpha/\beta$ hydrolase fold family. It has a typical structure of eight β-sheets connected by α-helices (Fig. 1(a)). The enzyme in its natural state is a monomer with a molecular weight of around 60,000 Daltons. It bears a striking resemblance to several hydrolase structures including dienelactone hydrolase, serine carboxypeptidase-II, three neutral lipases, and haloalkane dehalogenase **[24]**.

Determining protein–small molecule binding affinity is an important part of drug discovery. Molecular docking simulations are computational methods that predict small molecule-protein interactions by mathematical algorithms. In this study, Porphyrin derivatives: 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) (Fig. 3) were tested by Molecular Docking as inhibitors of human $AChE_H$ enzyme. The experimentally observed docking (activity) pattern in terms of stability of binding was found to be: TPPS > FeTPPS > FeNOTPPS. The strength of binding was quantified by use of a Scoring Function that approximates the free energy of binding.

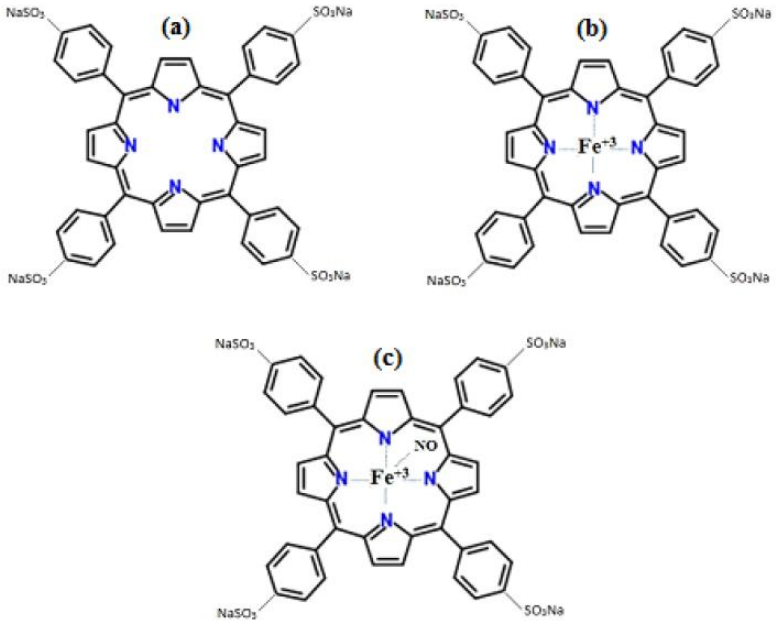


**Figure3.** *Chemical structures of TPPS, FeTPPS and FeNOTPPS*

**Table1.** *Breakup of energies of TPPS, FeTPPS and FeNOTPPS after energy minimization*

| Parameter | Energy (kcal/mol) | | |
|---|---|---|---|
| | **TPPS** | **FeTPPS** | **FeNOTPPS** |
| **Bond Stretching Energy** | 1506.6 | 352.2 | 325.7 |
| **Bond Stretching & Bending Energy** | 88.5 | -4.6 | -7.8 |
| **Torsional Energy** | 68.4 | 17.7 | 18.8 |
| **Out of Plane Bending Energy** | 84.4 | 22.2 | 19.5 |
| **Electrostatic Energy** | 1041.6 | 703.9 | 565.1 |
| **Angular Strain Energy** | 2682.8 | 1227.9 | 1126.3 |
| **Dihedral Interaction Energy** | 1181.9 | 622.7 | 574.5 |
| **Torsional Stretching Energy** | -91.5 | -59.2 | -56.1 |
| **Van der Waals Energy** | 4464.3 | 1626.9 | 1554.9 |
| **Hydrogen Bonding Energy** | 379.3 | -140.1 | -315.6 |
| **Total Energy** | **11406.1** | **4369.7** | **3805.4** |

The result produced by the study is actually the opposite of the earlier experimentally determined trend by enzymatic assays **[25]**. Most probably, the nature of the enzyme and the differential fluidity changes of the erythrocyte membrane is the causative factor for the inhibitory effect in the experimental results. These analogues can dock at a site of the cell membrane that is away from the active site and cause differential changes in the membrane properties which can, in turn, affect the enzymatic activity. However, these are both assumptions as it is hard to predict the exact mode of docking of such analogues on the erythrocyte

## 4. CONCLUSION

The Molecular Dynamics simulations showed that three porphyrin compounds, 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) are potential inhibitors of $AChE_H$ activity. Interaction between these compounds and aromatic amino acids lining the gorge allows these ligands to penetrate deeply into the anionic site and oxyanion hole regions all the way down to the choline-binding site. The study showed that the binding affinity of these derivatives for $AChE_H$ does not increase with the increase in hydrophobicity of the molecules.

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