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IN SILICO APPROACH TO DISCOVER THE ROLE OF METALS FOR THE TREATMENT OF ALZHEIMER'S DISEASE AMYLOID-BETA (Aβ) PEPTIDE

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ABSTRACT : Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder and is characterized pathologically by the presence of amyloid plaques, extensive neuronal death, and shrinkage of the brain. Amyloid precursor protein (APP) can function as a metalloprotein and modulate copper transport via its extracellular copper binding domain (CuBD). CuBD of Amyloid precursor protein (APP) can strongly bind Cu(II) and reduce it to Cu(I) this can lead to increased copper-mediated neurotoxicity in cultured neurons (White et al. 1999a), presumably through increased oxidative stress or the generation of reactive oxygen species.

Role of metal ions in drug targeted proteins such as copper, zinc, and calcium will be discovered in this work. Therefore, anti-aggregatory agents like metal chelators such as Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G have been used in our study which will remove metal ion and thus reducing aggregation of Aβ peptide.

In this proposed work, we will build quantitative structure-activity relationship (QSAR) models, with known experimentally verified inhibitors have inhibitory value IC_{50} against APP. We will dock these inhibitors at the active site of APP using AutoDock software or Molecular Virtual Docker (MVD), which results an energy-based descriptors for QSAR modeling. Multiple Linear Regression models will be generated using energy-based descriptors and inhibitory value IC_{50}, which yield correlation coefficient. Further, new derivatives of potent natural compounds will be identifying using PubChem compound database and dock with active site of APP protein structure. After docking, new compounds having lower binding energy even lower than standard control Drug with APP will be selected as potential candidate drugs for Alzheimer's.

Key words : Protein Data Bank, Alzheimer's, Rational Drug Design, Virtual Screening, docking simulation, quantitative structure-activity relationship (QSAR), chelators and Amyloid-beta (Aβ).

INTRODUCTION

A number of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease, amyotrophic lateral sclerosis, and prion disease have been closely linked to disturbances in copper homeostasis in the central nervous system and the brain (Strausak *et al*, 2001; Sayre *et al*, 2000). AD is the most common progressive neurodegenerative disorder and is characterized pathologically by the presence of amyloid plaques, extensive neuronal death, and shrinkage of the brain. Alzheimer's disease is the most common cause of dementia. Globally, more than 24 million people were suffering from dementia in 2005 (Ferri *et al*, 2005). Its pathological symptoms include forgetfulness and memory loss (Mattson, 2004). AD is characterized by the presence of Amyloid-beta (Aβ) peptide plaques in the brain (Masters and Beyreuther, 2006), as major source of the neurotoxicity in AD is due to damage of cultured neurons by the action of soluble Aβ oligomers (Crouch *et al*, 2008). The current line of treatment of AD only provides symptomatic relief (Davis and Powchik, 1995; Sugimoto *et al*, 1995). Drugs of purely curative or preventive type are still not marketed.

Commonly used drugs are Acetylcholine esterase inhibitors (Sugimoto *et al*, 1995), which temporarily alleviate symptoms by raising levels of neurotransmitter Acetylcholine and thus improving cognitive behavior. These drugs are associated with some adverse side effects

as well (Davis and Powchik, 1995). 42 amino acid form of Aβ had been identified as the predominant constituent of plaques (Yin *et al*, 2007, Maloney *et al*, 2014). Therefore a preventive and curative strategy must deal with the reduction in Aβ 42 production. Aβ peptides are generated by successive cleavages of amyloid precursor protein (APP) by β and γ secretase (Potter and Dressal, 2000) enzyme.

Amyloid precursor protein (APP) can function as a metalloprotein and modulate copper transport via its extracellular copper binding domain (CuBD). CuBD of Amyloid precursor protein (APP) can strongly bind Cu(II) and reduce it to Cu(I) in vitro (Hesse *et al*, 1994; Multhaup *et al*, 1996). This can lead to increased copper-mediated neurotoxicity in cultured neurons (White *et al*, 1999a), presumably through increased oxidative stress or the generation of reactive oxygen species (Multhaup *et al*, 1997). The interaction between copper ions and CuBD of Amyloid precursor protein can modulate Aβ production. The treatment of CHO cells overexpressing APP with extracellular Cu(II) leads to reduced Aβ production and a shift of the cleavage equilibrium away from the amyloidogenic pathway (Borchardt *et al*, 1999). The effects are abolished when the copper binding residues of CuBD are mutated (Borchardt *et al*, 2000). In addition to copper binding to CuBD, copper is also known to bind the Aβ peptide fragment of APP, inducing peptide aggregation and leading to the production of reactive oxygen species via Fenton chemistry (Smith *et al*, 2007). Aβ aggregation has been observed to be induced by copper ion (Barnham *et al*, 2004). Therefore, anti-aggregatory agents like metal chelators such as Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G have been used will remove metal ion and thus reducing aggregation of Aβ peptide (Hanson *et al*, 2007). Although metal chelators lead to behavioral improvements, but they have not emerged as preventive alternatives. In this project, we will describe intermolecular interaction energy calculations on amyloid beta peptide with anti aggregatory compounds such as Deferoxamine, Memantine, Curcumin, Clioquinol, Chrysamine-G, VK-28, HLA-20, MK-30 and NE-18. Energetics involved in metal ion induced self-aggregation of amyloid beta peptide indicates that metal induced self-assemblage of Aβ is highly favored. Any compound can act as an anti-aggregatory agent if it can compete with Aβ for metal ions. It is predicted that VK-28 will be a preventive compound compare to other compounds which can compete with Aβ for metal ions.

In this proposed work, we will use *in silico* chelating agents such as Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G (Yadav and Sonker, 2011) and 8-hydroxyquinoline analogues (Budimir, 2011) against amyloid beta peptide for their potential to treat neurodegeneration etc. (Yang *et al*, 2002). Comparision of these results with existing chelating agents therapeutically being used for AD, like VK-28, HLA-20, MK-30 and NE-18.

MATERIALS AND METHODS

Metal ion chelators can work as potential therapies for diseases involving metal ion imbalance. Neurodegeneration is an excellent target for exploiting the metal chelator approach to therapeutics. We have investigated *in silico* several chelating agents for their potential to treat neurodegeneration such as Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G (Yadav and Sonker, 2011) and 8-hydroxyquinoline analogues (Budimir,2011) such as VK-28, HLA-20, MK-30 and NE-18. Chemical structures of Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G are shown in Fig. 1. 2D structures of 8-hydroxyquinoline analogues are shown in Fig. 2.

Solution structure of the Alzheimer's disease amyloid beta-peptide (1-42) (PDB ID: 1IYT) was retrieved from Protein Databank (http://www.rcsb.org/). The 2D structures of Deferiprone, Memantine, Curcumin, Clioquinol and Chrysamine-G, VK-28, HLA-20, MK-30 and NE-18 were constructed using ChemSketch and converted into the 3D structure using OpenBabel (O'Boyle

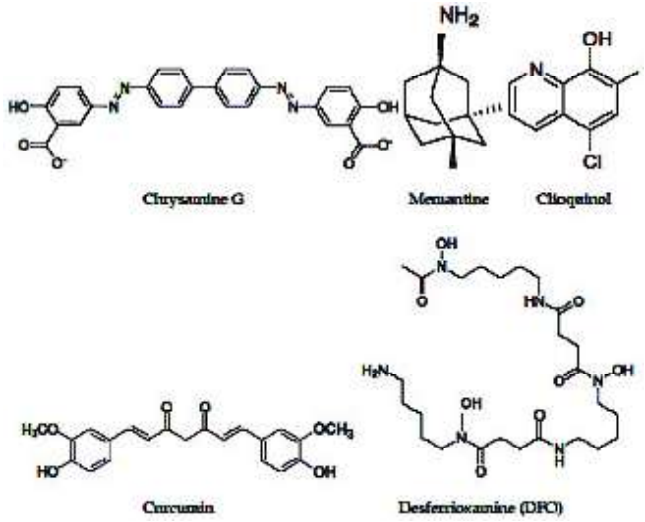

Fig. 1: Chemical structures for some selected Metal Chelators.

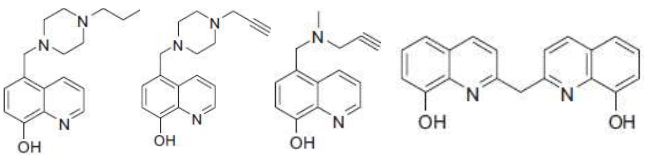

Fig. 2 : Chemical structure of Chelators tested in AD.

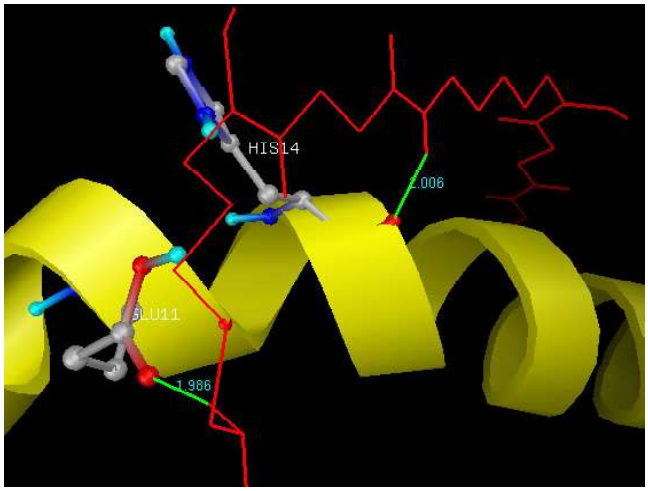

Fig. 3 : Metal chelation and intercalation of Aβ by Deferoxamine. Two h-bonds are formed between amino acid HIS14 (O) and GLU11 (OE1) of Amyloid Beta peptide (Pdb Id.: 1IYT) with Deferoxamine (H68),(H84), respectively. Inhibitor Deferoxamine is show in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

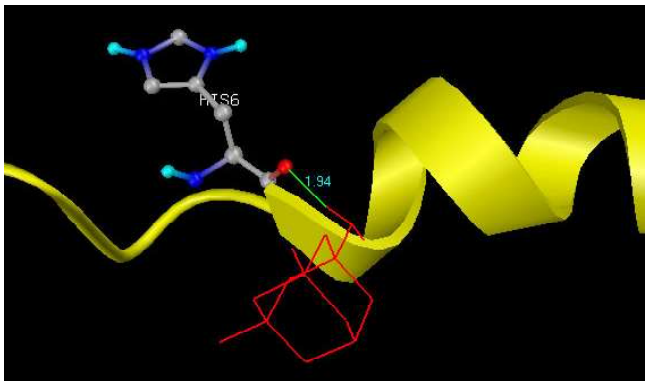

Fig. 4 : Metal chelation and intercalation of Aβ by Memantine. One h-bond is formed between amino acid HIS6 (O) of Amyloid Beta peptide (Pdb Id.: 1IYT) with Memantine (H). Inhibitor Memantine is shown in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

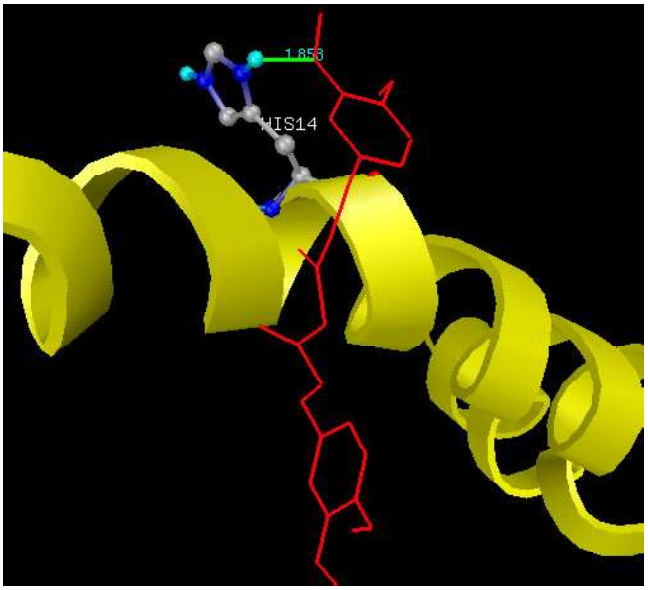

Fig. 5 : Metal chelation and intercalation of Aβ by Curcumin. One h-bond is formed between amino acid HIS14 (HD1) of Amyloid Beta peptide (Pdb Id.: 1IYT) with Curcumin (O). Inhibitor Curcumin is show in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

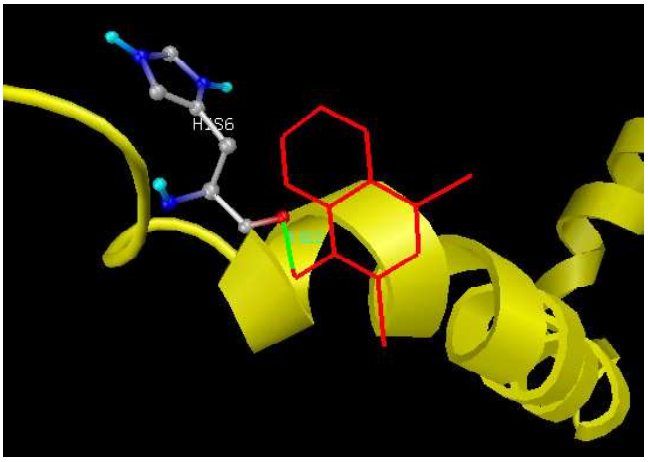

Fig. 6 : Metal chelation and intercalation of Aβ by Clioquinol. One h-bond is formed between amino acid HIS6 (O) of Amyloid Beta peptide (Pdb Id.: 1IYT) with Clioquinol (H), respectively. Inhibitor Clioquinol is show in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

et al, 2011) tool. All the compounds were subjected to energy minimization and molecular dynamics using the HyperChem software (HyperChem Release 7.5). Energy calculations were carried out using the AMBER force field. Molecular structure optimization of ligands was carried out using conjugate gradient method Polak-Ribiere until the maximum energy derivative was lower than 0.1kcal/Å mol in order to obtain a correct geometry.

Molecular docking

Docking of Metal Chelators with amyloid beta (Aβ) peptide structure was carried out using AutoDock v3.0.5 (Goodsell *et al*, 1996; Morris *et al*, 1998). Gasteiger charges were added to the compound, and maximum 6 number of active torsion are given to the lead compound using AutoDock Tool (http://autodock.scripps.edu/resources/adt). Kollaman charges and solvation term were added to the protein structure using AutoDock Tool. The Grid for docking calculation was centered to cover the protein binding site residues and accommodate compound to move freely. During the docking procedure, a

Table 1 : The docking results of metal chelators with Amyloid Beta peptide.

S. No.	CID No	Inhibitor name	Binding Energy (Kcal/mol)	Docking Energy (Kcal/mol)	IntermolEnergy (Kcal/mol)	Torsional Energy (Kcal/mol)	Internal Energy (Kcal/mol)
1	2973	Deferoxamine	2.28	-6.39	-6.43	8.72	0.05
2	4054	Memantine	-6.5	-6.52	-6.5	0.0	-0.01
3	969516	Curcumin	-3.71	-3.35	-6.2	2.49	2.85
4	2788	Clioquinol	-4.47	-4.51	-4.47	0.0	-0.04
5	6913223	Chrysamine-G	-4.34	-6.39	-6.21	1.87	-0.19
6	-	VK-28	-5.73	-7.22	-6.98	1.25	-0.25
7	6918819	HLA-20	-7.39	-8.58	-8.63	1.25	0.05
8	6918822	MK-30	-5.08	-6.34	-6.32	1.25	-0.02
9	-	NE-18	-5.9	-6.48	-6.52	0.62	0.04

Lamarckian Genetic Algorithm (LGA) were used for flexible ligand rigid protein docking calculation. Docking parameters were as follows: 30 docking trials, the population size of 150, the maximum number of energy evaluation ranges of 25,0000, the maximum number of generations is 27,000, the mutation rate of 0.02, cross-over rate of 0.8. Other docking parameters were set to the software's default values.

RESULTS AND DISCUSSION

Metal chelators will remove metal ion and thus reducing aggregation of Aβ peptide (Hanson *et al*, 2007). Although, metal chelators lead to behavioral improvements, but they have not emerged as preventive alternatives. Energetic aspects related to inefficiency of these drugs have been studied in this chapter.

A compound may act as anti aggregatory in two possible ways: (i) By removing toxic metal ions from brain. (ii) By intercalating on to Aβ and masking its portion actively involved in aggregation. Any attempt at designing anti aggregatory compound is bound to succeed, if we first try to understand why metal chelator drugs used for removal of metal toxicity in the past are not so effective in the present case. For a compound to be an efficient anti-aggregation agent, it must be sufficiently competitive with metal ion for interaction at Aβ. Deferoxamine, Memantine, Curcumin, Clioquinol and Chrysamine-G and 8-hydroxyquinoline analogues are known to possess anti-aggregation property as well as brain permeability. Docking studies have been performed to study energetics involved in self aggregation of Aβ and drugs. AutoDock tool have been used for flexible ligand docking studies and generating poses for drug interacting with Aβ. Drugs were docked with amyloid beta-peptide in different poses using AutoDock tool. Docking energies of these drugs with Aβ peptide were shown in Table 1.

Docking pose of the best confirmation of Deferoxamine in the binding site of amyloid beta (Aβ) peptide is shown in Fig. 3. Deferoxamine forms two hydrogen bonds with hydrophobic amino acid Histidine and negatively Charged Glutamic Acid of Amyloid Beta peptide. Residues of a protein involved in the formation of hydrogen bonds with this compound are represented as sticks and balls model. Binding site residues of Cu (II) bound to Aβ (1-16) structure consist of HIS6, HIS14, HIS13, and GLU11 (Streltsov *et al*, 2008). Therefore, Deferoxamine docked with amyloid beta (Aβ) peptide at same binding pocket. Deferoxamine has docking energy of -6.39 kcal/mol and can competitively inhibit metal-induced aggregation by removing metal ion toxicity.

Docked complex of Memantine with amyloid beta (Aβ) peptide is shown in Fig. 4. Memantine form one hydrogen bond with hydrophobic amino acid Histidine of Amyloid Beta peptide. Residues of a protein involved in the formation of hydrogen bonds with this compound are represented as sticks and balls model. Binding site residues of Cu (II) bound to Aβ (1-16) structure consist of HIS6, HIS14, HIS13, and GLU11 (Streltsov *et al*, 2008). Therefore Memantine docked with amyloid beta (Aβ) peptide at similar binding pocket as copper ion bound with Aβ (Streltsov *et al*, 2008). Memantine has docking energy of -6.52 kcal/mol and can competitively inhibit metal-induced aggregation by removing metal ion toxicity.

Docking pose of the best confirmation of Curcumin in the binding site of amyloid beta (Aβ) peptide is shown in Fig. 6. Curcumin forms one hydrogen bond with hydrophobic amino acid Histidine. Curcumin docked with a same binding pocket of copper as HIS6, HIS14, HIS13, and GLU11 with Aβ peptide (Maloney and Streltsov *et al*, 2008). On the basis of docking energy, it is predicted that Curcumin (docking energy: -3.35 kcal/mol) will not be sufficiently competitive with Aβ for metal ions.

Interaction of Clioquinol with amyloid beta (Aβ) peptide is shown in Fig. 7. Clioquinol form one hydrogen bond with Histidine residue of Amyloid Beta peptide. Clioquinol has docking energy of -4.51 kcal/mol with Aβ and is predicted that it will not be sufficiently competitive

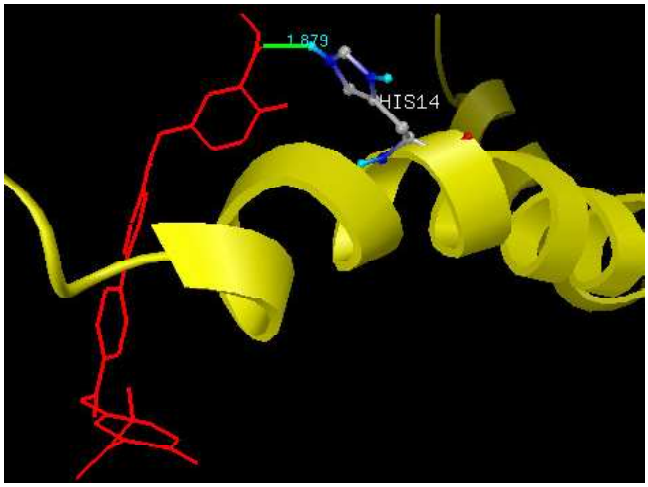

Fig. 7 : Metal chelation and intercalation of Aβ by Chrysamine-G. One h-bond is formed between amino acid HIS14 (HE2) of Amyloid Beta peptide (Pdb Id.: 1IYT) with Chrysamine-G (O). Inhibitor Chrysamine-G is shown in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

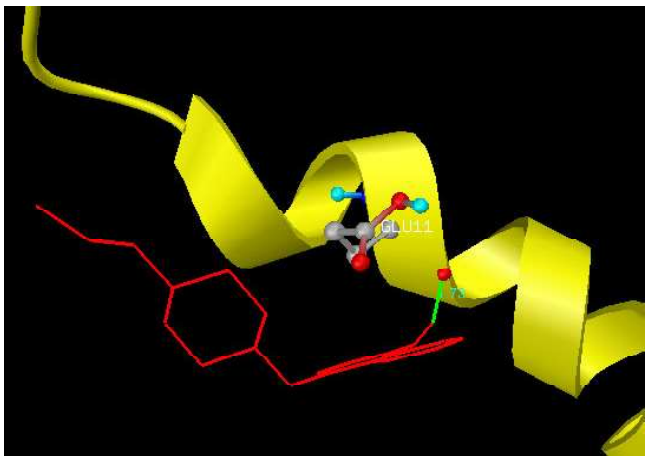

Fig. 8 : Metal chelation and intercalation of Aβ by VK-28. One h-bond is formed between amino acid GLU11 (O) of Amyloid Beta peptide (Pdb Id.: 1IYT) with VK-28 (H12). Inhibitor VK-28 is shown inline representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

with Aβ for metal ions.

Best docked pose of Chrysamine-G in the binding site of amyloid beta (Aβ) peptide is shown in Fig. 8. Chrysamine-G form one hydrogen bond with hydrophobic amino acid Histidine of Amyloid Beta peptide. Chrysamine-G has simillar binding pocket residues HIS6, HIS14, HIS13, and GLU11 as Cu (II) has with Aβ peptide. Chrysamine-G has docking energy of -6.39 kcal/mol with Aβ and can competitively inhibit metal-induced aggregation by removing metal ion toxicity.

Docking pose of the best conformation of VK-28 in

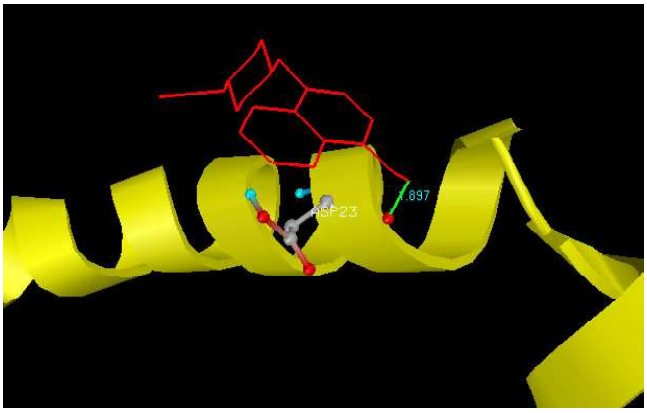

Fig. 9 : Metal chelation and intercalation of Aβ by HLA-20. One h-bond is formed between amino acid ASP23 (O) of Amyloid Beta peptide (Pdb Id.: 1IYT) with HLA-20 (H). Inhibitor HLA-20 is shown inline representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

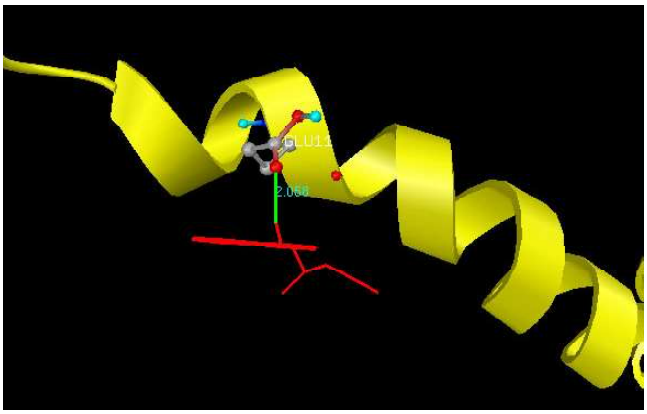

Fig. 10: Metal chelation and intercalation of Aβ by MK-30. One h-bond is formed between amino acid GLU11 (OE1) of Amyloid Beta peptide (Pdb Id.: 1IYT) with MK-30 (H). Inhibitor MK-30 is show in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

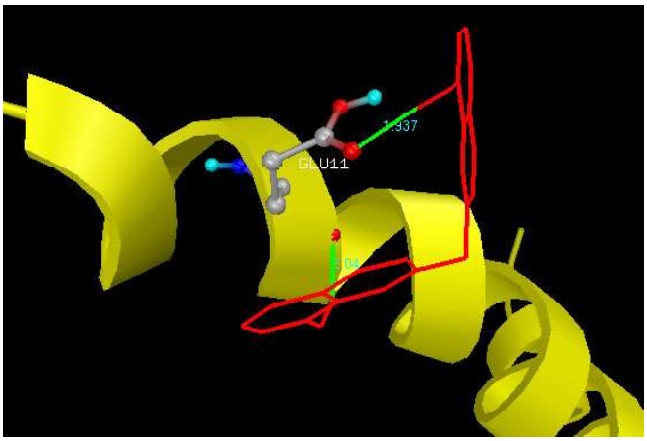

Fig. 11 : Metal chelation and intercalation of Aβ by NE-18. Two h-bonds are formed between amino acid GLU11 (OE1) and GLN11 (O) of Amyloid Beta peptide (Pdb Id.: 1IYT) with NE-18 (H25), (H23), respectively. Inhibitor NE-18 is shown in line representation and is colored with red using Python Molecular Viewer. Amino acid residues are representation as sticks and balls. Hydrogen bond is represented by green line.

binding pocket of amyloid beta (Aβ) peptide is shown in Fig. 9. VK-28 form one hydrogen bond with negatively charged Glutamic Acid of Amyloid Beta peptide. VK-28 has docking energy of -7.22 kcal/mol and can competitively inhibit metal induced aggregation by removing metal ion toxicity.

The putative second binding site of Cu (II) from the Aβ (18-41) tetramer crystal structure consist of ASP23 and GLU22 (Streltsov *et al*, 2011). HLA-20 was docked with Aβ peptide at the putative second binding site of Cu (II) from the Aβ (Streltsov *et al*, 2011). HLA-20 (docking energy: -8.58 kcal/mol) will not be sufficiently competitive with Aβ for metal ions.

Best docked pose of MK-30 in binding site of amyloid beta (Aβ) peptide is shown in Fig. 10. Chrysamine-G form one hydrogen bond with polar amino acid Glutamic Acid of Amyloid Beta peptide. Chrysamine-G has simillar binding pocket residues HIS6, HIS14, HIS13, and GLU11 as Cu (II) has with Aβ peptide. Chrysamine-G has docking energy of -6.34 kcal/mol with Aβ and can competitively inhibit metal induced aggregation by removing metal ion toxicity.

The docked pose of MK-30 in the binding pocket of amyloid beta (Aβ) peptide is shown in Fig. 11. Chrysamine-G form one hydrogen bond with polar amino acid Glutamic Acid of Amyloid Beta peptide. NE-18 (docking energy: -6.48kcal/mol) can competitively inhibit metal-induced aggregation by removing metal ion toxicity.

It is understood that efficiency of preventive drugs depends on their ability to compete with Aβ for metal ions which is extremely difficult as Aβ has very high affinity for metal ions. By this understanding, it is predicted that VK-28 will be a preventive compound compared to other compounds which can compete with metal ions for Aβ and can also intercalate to Aβ.

CONCLUSION

Diverse experimental techniques have been utilized over the past few years in elucidating the nature of the interactions of transition metal ions with the amyloid beta peptide. Much of it has concerned copper, in both its oxidation states, due to its redox activity and biological relevance. It is clear that the interactions are complex, and very sensitive to ambient conditions. This makes it quite challenging to discern the interactions that are occurring in the brain. As of yet, there is no x-ray crystallographic structure of a metal ion complexed with Aβ. Such structures could provide templates for the design of therapeutics and diagnostics for AD. In this chapter, we describe intermolecular interaction energy calculations on amyloid beta peptide with anti aggregatory compounds as Deferoxamine, Memantine, Curcumin, Clioquinol, Chrysamine-G, VK-28, HLA-20, MK-30 and NE-18. Energetics involved in metal ion induced self-aggregation of amyloid beta peptide indicates that metal induced self-assemblage of Aβ is highly favored. Any compound can act as an anti-aggregatory agent if it can compete with Aβ for metal ions. It is predicted that VK-28 will be a preventive compound compare to other compounds which can compete with Aβ for metal ions.

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