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Investigation of Mutant GFAP Protein Associated with Alexander Disease and Its Therapeutic Intervention: Structure Based Drug Design Approach

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Abstract

Systems Biology approach involves integration of experimental and computational research to understand complex biological systems. Alexander's Disease (AxD) was first described by W. S. Alexander in 1949, and is a rare, but often fatal neurological disorder that has been divided into three subtypes based on the age of onset: the infantile, juvenile and adult forms that are shown to be caused by mutations in the gfap gene. The infantile form, with onset between birth and about two years of age, is currently the most common form of the disease. The characteristic neuropathological feature of all forms of AxD is the presence of Rosenthal fibers. In present study, the mutant GFAP protein associated with AxD was investigated by predicting the structure of wild type and mutant GFAP protein. It was found that due to the reported single point mutation, even though both the wt and mutant GFAP proteins adopt a right handed alpha helix structure but large number of residues in the mutant GFAP were spread in the region of left-handed helix and beta sheets in the Ramachandran Plots. This resulted in a large conformational change which may be responsible for the cause of aggregation of mutant GFAP forming Rosenthal Fibers. In the absence of any commercially available drug to alleviate the symptoms of AxD, the therapeutic intervention of mutant GFAP protein was done using structure based drug design approach. The drug dibutyryl cyclic AMP identified through data mining from STITCH 4.0 was found to be toxic and therefore its structural analogs were generated using GAUSSIAN 09. Each of the 20 structural analogs of dbcAMP were docked with mutant GFAP using Discovery Studio 2.5 and analyzed for their toxicity potential using OSIRIS Property Explorer. Two structural analogs i.e. DBCM17 and DBCM20 were found to have favorable docking, druglikeness and did not pose any toxicity risk. These structural analogs identified may be further analyzed for therapeutic intervention of AxD by their role in prevention of aggregation of mutant GFAP.

Keywords: In Silico; Structure Based Drug Design; Alexander Disease; Point Mutation; Rosenthal Fibers.

1. Introduction

Computational biology, through pragmatic modeling and theoretical exploration, provides a powerful foundation to formulate and solve critical biological problems [1]. Alexander disease (AxD), also known as fibrinoid leukodystrophy, is a progressive and fatal neurodegenerative disease that affects the midbrain and cerebellum of the central nervous system. It is a rare genetic disorder that mostly affects infants and children, causing developmental delay and changes in physical characteristics. The destruction of white matter in the brain is accompanied by the formation of fibrous, eosinophilic deposits known as Rosenthal fibers [2]. Rosenthal fibers appear not to be present in healthy people, but occur in specific diseases, like some forms of cancer. They are aggregations of protein that occurs in astrocytes, which are supporting cells of the brain. These aggregates are found in other disorders, but not with the abundance of particular distribution in the brain that occurs in AxD. Mutations in the coding region of gfap that codes for glial fibrillary acidic protein (GFAP) which maps to chromosome 17q21 [3] and have been shown to contribute to the accumulation of Rosenthal fibers [4]. Most of the gfap mutations that have been identified in AxD are heterozygous, sporadic and missense mutations as reported by Nam et al. (2015).

Analysis of the proband's gfap revealed a heterozygous G to T substitution in exon 6 at position 934, causing an amino acid change at codon 312, a G to T change converts a glutamate GAG codon to a TAG nonsense codon, resulting in deletion of 121 amino acids [5]. Like other intermediate filament proteins, GFAP consists of a central four-part alpha helical rod segment flanked by N-terminal head and C-terminal tail random coils. The mutation leads to the deletion of rod domain and whole tail domain in the GFAP. The tail domain is critical to the proper assembly of GFAP, the mutated gfap could not form proper GFAP networks, thereby leading to the formation of aggregates [6].

The current standard of treatment for AxD is symptomatic and focuses on major problems such as seizure control, nutrition, and maintenance of pulmonary function. Only three reports describe attempts at alternative forms of therapy. One patient, studied prior to the discovery of gfap mutations as the cause of the disease, was given bone marrow transplantation based on the mistaken analogy to other leukodystrophies that are treated in such a manner that the patient died 4.5 months after transplantation, at the age of 1 year [7].

Therefore in the absence of any therapy/ commercially available drug for treatment of AxD or alleviation of its neuropathological feature of aggregation of Rosenthal Fibers, the present study was undertaken. The aim of the present work is to predict the structure of wt GFAP protein, its mutant and to further develop a potential therapeutic agent which may prevent the aggregation of mutant GFAP protein.

2. Methodology

2.1. Structure Modeling

The GFAP protein sequence of Homo sapiens was retrieved from UniProt with ID P14136. The structure of glial fibrillary acidic protein (humans) was not available in PDB therefore it was predicted using I-TASSER which is an integrated platform for automated protein structure and function prediction based on the sequence-to-structure-to-function paradigm [8].

Verify3D was used for quality assessment of the structure. It determines the compatibility of an atomic model (3D) with its own amino acid sequence (1D) by assigning a structural class based on its location and environment (alpha, beta, loop, polar, nonpolar etc.) and comparing the results to valid structures. The vertical axis in the plot represents the average 3D-1D profile score for each residues in a 21-residue sliding window and the scores ranges from -1 (bad score) to +1 (good score) [9].

Also, Ramachandran Plot was constructed using Swiss-PDB Viewer to check the allowed and disallowed regions of torsion angle values, serving as an important indicator of the quality of protein three-dimensional structures torsion angle values [10].

2.2. Structure Based Drug Design

No commercially available drug has been reported for treatment of AxD therefore, STITCH 4.0 was used to identify the ligands for Homo sapiens GFAP and their structure was obtained from PubChem.

Osiris Property Explorer and Toxicity Checker was used to check the toxicity risk assessment and other parameters such as the drug score which combines druglikeness, cLogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the compound's overall potential to qualify for a drug [11].

Structural analogs were generated in GaussView module available in GAUSSIAN 09. The GaussView has the Clean menu which adjust the geometry of the drawn molecule, based on a defined set of rules, to more closely match chemical intuition and thus making respective structure stable [12].

Docking was performed using LigandFit module of Discovery Studio 2.5. The method employs a cavity detection algorithm for detecting invaginations in the protein as candidate active site regions. Candidate poses are minimized in the context of the active site using a grid-based method for evaluating protein-ligand interaction energies. Errors arising from grid interpolation are dramatically reduced using a new non-linear interpolation scheme [13].

3. Results

Alexander Disease (AxD) is caused by mutations in gfap that encodes a type III intermediate filament predominantly found in astrocytes within the CNS. AxD is caused by the heterozygous mutations in the gene for glial fibrillary acidic protein, due to which prominent protein aggregates inside astrocytes are formed. Loss of myelin and oligodendrocytes is also observed along with neuronal degeneration [14].

3.1. Structure Modeling

Structure of wild type GFAP protein or mutated GFAP resulting from deletion of 121 amino acids [10] was not available in the protein structure database. Therefore, to predict the structure of wild type and mutant protein the

sequence of GFAP of Homo sapiens was retrieved from UniProt having ID P14136 in the FASTA format. The sequence of mutated protein was obtained by removing 121 residues from the C- terminal of the wt protein which corresponds to mutation at 312 codon reported by Nam et al. (2015) [5].

The 3D structure of wild-type GFAP and mutant type was predicted using I-TASSER which is top ranked in CASP7 to CASP11 [15]. I-TASSER model with the highest confidence scores for wt GFAP and mutant GFAP were selected for further analysis (figure 1A and 1B).

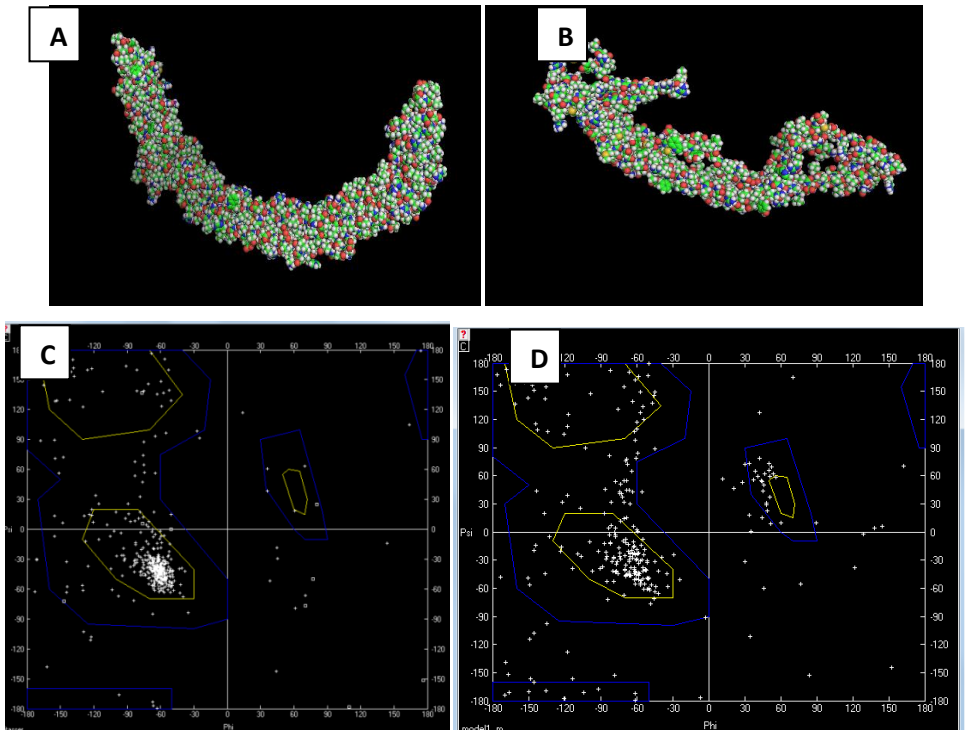


Figure 1: The structure of (A, C) wild type GFAP (432 residues) and (B, D) mutated GFAP (312 residues) predicted by I-TASSER and their corresponding Ramachandran Plots

On analysis of the Ramachandran Plot of wt GFAP and mutant GFAP, it was observed that the wt protein adopts a right handed alpha helix structure. But the residues in the mutant GFAP were also spread in the region of left-handed helix and beta sheets resulting in change of structure and distortion of the protein due to deletion of 121 amino acid residues following the point mutation in the gene at codon 312 as compared to the wt GFAP as can be seen from figure 1. The secondary structure element of α-helix is a common structural feature of proteins forming plaques in other neurogical diseases such as Parkinson's Disease, Huntington's Disease [16] and Prion Disease [17] as also in mutant GFAP protein of AxD. The distortion of mutant GFAP in comparison to the wt is also observed on superimposition of the two structures with an RMSD of 1.375Å.

From the results of Verify3D it was observed that in the wt-GFAP graph residue number 1-70, 90-130, 170-185, 230-290 and 300-400 are well predicted as they have score near to +1 (shown in figure 2A) and there are few residues that have not predicted reliably such as 70-90, 130-170, 185-230, 290-300 and 400-420 having score less than zero. On the other hand, in the mutant GFAP graph the residue numbers 20-120, 180-255, 270-290 were conjectured well having score greater or equal to 1 whereas residue number such as 1-20, 120-180, 260-270 and 290-300 were not reliably predicted (shown in figure 2B).

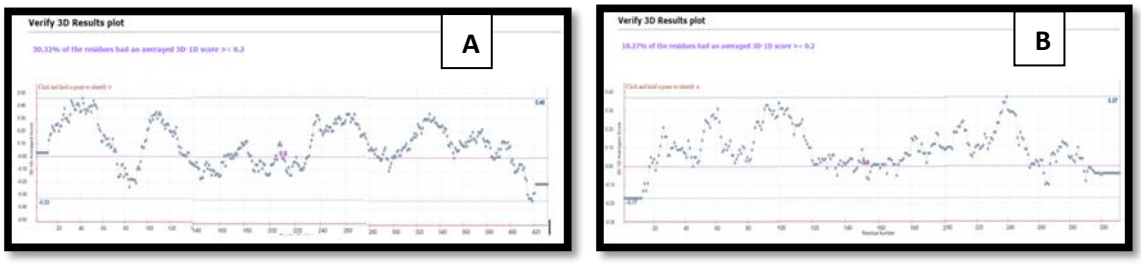


Figure 2: Verify3D validation of (A) wild-type GFAP and (B) mutant GFAP

3.2. Structure based drug design

In the absence of any commercially available drug reported for GFAP. The ligands for Homo sapiens GFAP were retrieved using STITCH 4.0 and the chemical compound dibutyryl cyclic AMP was found to be interacting with GFAP (Figure 3).

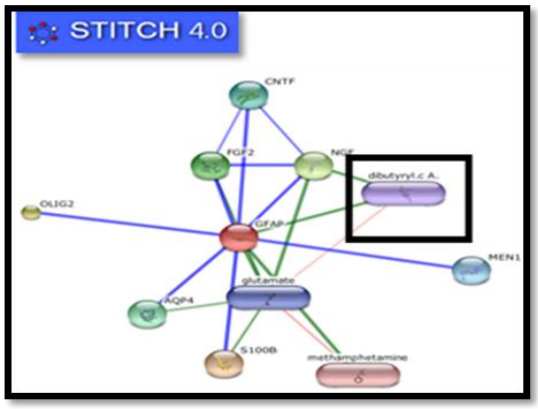
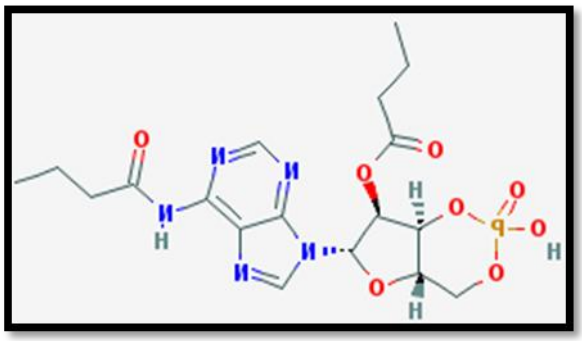


Figure 3: Ligand retrieved for GFAP using STITCH 4.0.

The drug dibutyryl cAMP (CID: 2460) has been reported to be used for the treatment of hepatocellular carcinoma [18]. Its structure and SMILES representation were retrieved from PubChem.



PubChem CID: 2460
CCCC(=O)NC1=NC=NC2=C1N=C N2C3C(C4C(O3)COP(=O)(O4)O)O C(=O)CCC

On investigation of dibutyryl cAMP for its toxicity risk using OSIRIS Property Explorer it was found to have unknown chirality and no results were obtained and therefore was further checked with Toxicity Checker and it was found to be toxic. Due to the toxic nature of dbcAMP it cannot be further investigated for its potential as a therapeutic agent. Therefore, twenty structural analogs were constructed in Gaussian 09 software by modification of dbcAMP.

All the 20 compounds were checked for their toxicity risks using OSIRIS Property Explorer and were docked with mutant GFAP using Discovery Studio 2.5 (Table 1). None of the analogs showed any toxicity risk analyzed in terms of mutagenecity, tumorogencity, irritability and reproductive effects. Out of the 20 analogs it was found that the compounds DBCM12, DBCM17 and DBCM20 (figure 4) had docking scores greater than dbcAMP docked with mutant GFAP and also greater than the scores of other variants (Table 1). The docking of dbcAMP and analog DBCM20 with mutant GFAP and its corresponding binding residue are shown in figure 5. From table 2 it was observed that amino acid residues **LEU90 GLU91, ASN94, LEU193, ILE197, HIS251, GLU254, and GLU255**

were common in the binding pocket of mutant GFAP and were surrounding the ligands namely DBCM12, DBCM17 and DBCM20 respectively.

Even though DBCM12 had the highest dock score, its druglikeness was negative and it possessed the lowest drug score (OSIRIS Property Explorer) thereby making it unsuitable as a potential drug. Therefore, the analog DBCM20 having a high druglikeness and dock score (Discovery Studio 2.5) followed by DBCM17 are potential therapeutic agents. These may be further investigated for their therapeutic potential to treat AxD by prevention of aggregation of Rosenthal Fibers.

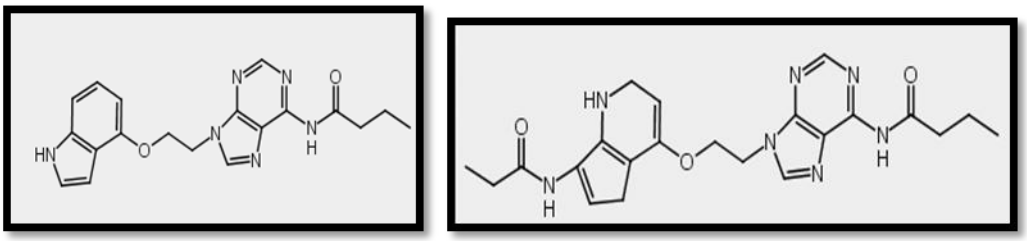


Figure 4: Structure of (A) DBCM12 and (B) DBCM20

Table1: Docking scores, toxicity risk and drug-likeness parameters of dibutyryl cAMP (dbcAMP) and its structural analogs

Variants	Dock score	TR*	cLogP	logS	M.W.	TPSA	DL*	Drug Score
dbcAMP	54.41	Yes	-	-	-	-	-	-
DBCM1	30.28	No	1.08	-2.74	219	72.7	-2.03	0.52
DBCM2	33.33	No	1.22	-0.88	240	59.81	0.69	0.8
DBCM3	34.19	No	1.27	-3.24	237	98	-0.33	0.54
DBCM4	30.27	No	1.03	-0.9	236	93.95	-1.15	0.6
DBCM5	34.65	No	1.66	2.49	262	59.81	-0.09	0.71
DBCM6	36.12	No	1.47	0.07	351	72.7	2.07	0.86
DBCM7	37.14	No	1.47	0.07	307	72.7	2.07	0.89
DBCM8	33.70	No	1.1	-2.71	235	92.93	0.7	0.77
DBCM9	40.35	No	2	-2.93	262	80.04	1.71	0.83
DBCM10	43.95	No	2.35	-3.69	325	81.93	-0.94	0.52
DBCM11	48.94	No	2.95	-4.43	359	81.93	-1.24	0.44
DBCM12	99.51	No	2.39	-4.22	364	97.72	-0.98	0.48
DBCM13	38.03	No	0.87	-1.22	267	89.77	2.8	0.93
DBCM14	49.89	No	3.19	-4.93	381	110.1	-0.65	0.43
DBCM15	52.17	No	1.64	-4.16	384	122.2	0.13	0.58
DBCM16	54.28	No	3.12	-4.41	456	93.96	0.09	0.48
DBCM17	56.02	No	2.89	-4.28	456	93.96	0.49	0.52
DBCM18	55.59	No	3.35	-4.55	422	93.96	-3.08	0.33
DBCM19	50.05	No	2.12	-3.51	435	117.8	0.19	0.58
DBCM20	59.60	No	1.74	-4.22	437	123.0	1.04	0.60

TR* - Toxicity Risks; DL* - Druglikeness

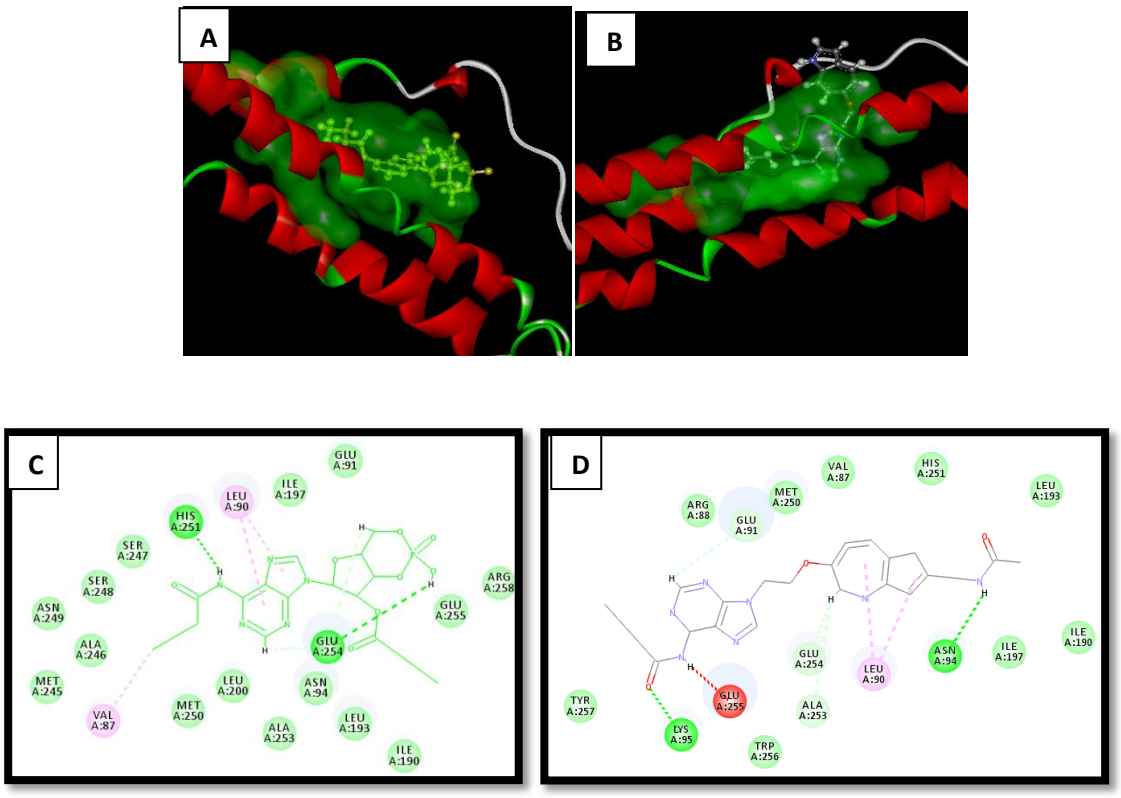


Figure 5: Docking and binding site residues of mutant GFAP with (A, C) dbcAMP and (B, D) DBCM20 Binding site residues of GFAP with dbcAMP.

Table 2: Binding pocket residues interacting with dbcAMP and analogs

Compound	**Binding pocket residues**	**No. of H-bonds**	**Residues exposed to solvent**
dbcAMP	VAL87, **LEU90, GLU91, ASN94, LEU193, ILE197**, ILE190, LEU200, MET245, ALA246, SER247, SER248, ASN249, MET250, **HIS251,** ALA253, **GLU254, GLU255** and ARG258	2 (HIS251 and GLU254)	LEU90, ASN94, LEU193, HIS251, and GLU254
DBCM12	VAL87, **LEU90, GLU91, ASN94,** LYS95, **LEU193, ILE197,** LEU200, MET250, **HIS251**, ALA253, **GLU254, GLU255** and TYR257	1 (**GLU255**)	VAL87, LEU90, GLU91, ASN94, LYS95, LEU193, ILE197, MET250, HIS251, GLU254, GLU255 and TYR257
DBCM17	**LEU90, GLU91, ASN94,** LYS95, **LEU193, ILE197, HIS251, GLU254, GLU255** and TYR257	1 (**ASN94**)	LEU90, GLU91, LYS95, ASN94, LEU193, ILE197, HIS251, GLU254 and GLU255
DBCM20	VAL87, ARG88, **LEU90, GLU91, ASN94,** LYS95, ILE190, **LEU193, ILE197,** MET250, **HIS251,GLU254,** ALA253, **GLU255,** TRP256 and TYR257	3 (ASN94, GLU254 and **GLU255**)	VAL87, ARG88, LEU90, GLU91, ASN94, LYS95, ILE197, HIS251, MET250, GLU254, and GLU255

4. Conclusion

Based on the docking results it can be concluded that the drug obtained through data mining from STITCH 4.0 dibutyryl cAMP (dbcAMP) cannot be used therapeutically due to its high toxicity. Therefore 20 structural analogs were generated and analyzed using in silico tools. The structural analogs DBCM17 and DBCM20 were found to have favorable docking scores, druglikeness and did not pose any toxicity risk. These compounds may further be investigated for their efficacy for treatment of Alexander Disease to alleviate the symptoms associated with AxD and prevent aggregation of mutant GFAP that form Rosenthal Fibers.

5. List of Abbreviations

AxD: Alexander Disease, GFAP: Glial fibrillary acidic protein, dbcAMP: dibutyryl cyclic adenosine monophosphate, TR: Toxicity Risks, DL: Druglikeness.

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Conflict of Interests

Authors have declared that no conflict of interest exists.

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