CHAPTER 7

Deciphering the role of Chameleon fragments in the folding of Amyloidogenesis
CHAPTER VII

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7.1. Introduction

Proteins are dynamic macromolecules and it is believed for most proteins, amino acid sequence determines the tertiary structure which eventually responsible for function (Dill, K et al., 2012), (Kabsch et al., 1984). Secondary structural elements such as helices and beta strands are building blocks for protein tertiary structure (Chakrabartty et al., 1995) (Jones et al., 1999) and the formation of these secondary structural elements is the result of a combination of local and non-local interactions (Frishman et al., 1996) (Saravanan, KM et al., 2010). Proteins are stabilized by different biophysical forces and they can alter the formation of their secondary structures upon various factors (Minor Jr et al., 2010). Number of methods and analysis have been reported in literature to identify flexible sequence fragments (Pongor et al., 1985) (Yoon et al., 2006) (Mutter et al., 1990) (Argos et al., 1987) (Sudarsanam S et al., 1998) (Jacoboni et al., 2000) (M Saravanan, K et al., 2012, 2013). With a high conformational degree of freedom these conformation changes are shown to be responsible for more than 30 human diseases associated with fibril formation and direct implications are evident in Alzheimer’s, Parkinson disease etc., (Egorov, V et al., 2015).

Amyloid is an extracellular protein which induces conformational transition of the constituent proteins into a β-sheet rich filament (Nelson et al., 2005). The protein
fragments with alpha-helix-forming propensity could lead to transition into a beta conformation and follow by the formation of amyloid fibrils (Takahashi, Yuta et al., 2000). It has been shown that some ionic self-complementary motifs with complimentary charged residues periodically arranged within the protein sequence could be capable of conformational transitions (Farnsworth, PN et al., 2000). During the process of amyloid aggregation, peptides undergo a conformational change to form typical structure dubbed as cross-β-sheets to break the globular symmetry of the molecule and gives rise to linear assemblies of ordered fibers (Ranganathan, S et al., 2016). Parallel studies too have confirmed that the conformational changes in proteins/peptides followed by amyloid aggregation also plays a pivotal role in the functions like cell signaling and many physiological processes in the cell (Majumdar, A et al., 2012) (Moresco, E et al., 2011)

In the context of current literature, it is necessary to understand the conformational switching phenomenon of the amyloid peptides by investigating the role of amino acid residues in terms of individual and segmental properties. In the present study, we explicitly account for a key feature in amyloid aggregation (conformational switch phenomenon) by computing secondary structural propensities of amino acid residues in amyloid beta peptides. In order to explore the secondary structure of identical fragments of amyloid beta peptide with different proteins, heuristic sequence search has been performed within whole Protein Data Bank (Berman, HM. et al., 2000). Further our study corroborates about the unfolding simulation of shark protein to understand the conformational switching.

7.2. Materials and Methods

We have considered the crystal structure of amyloid beta (18-41) peptide fusion with antigen receptor variable domain from sharks. The crystal structure PDB ID
(3MOQ) at 2.05 Å shows the presence of the tetrameric state. The sequence and three-dimensional coordinates of the above entry are retrieved from Protein Data Bank (PDB). Overlapping five residue fragments (pentapeptides) in amyloid β-peptide is considered and subjected to heuristic sub-sequence search against the nonredundant sequences in the PDB. Since, a stretch of five residues is enough to form a complete helix or β-strand, we considered five residue fragments were considered. The propensity of residues to adopt a helix or strand is computed by using the Chou-Fasman method. Chou and Fasman algorithm employs the grouping of twenty amino acid residues as strong helix formers, weak helix formers, strong beta former and weak beta former respectively. Dihedral angles of amino acid residues in the amyloid beta peptide are also computed. Further, the protein structure “3MOQ” is subjected to thermal unfolding simulation experiments by using Constraint Network Analysis (CNA) web server and fold amyloid program is used to identify the aggregation-prone regions in the protein. The CNA web server provides a refined modeling of thermal unfolding simulations that consider the temperature dependence of hydrophobic tethers and computes a set of global and local indices for quantifying bio macromolecular stability.

7.3. Results and Discussion:

Considering the beta-amyloid peptide, we have generated twenty overlapping pentapeptides to perform heuristic subsequence search in PDB entries. The sequence search results are shown in Table 7.1. The first column of table 7.1 represents the pentapeptide fragment of beta-amyloid along with DSSP assigned secondary structure. Other columns of the same table indicate identical pentapeptides in other unrelated proteins along with DSSP assigned secondary structure. Based on our heuristic subsequence search, we found a novel pentapeptide (highlighted pentapeptide in Table 7.1):
VLRDA) shows the completely different secondary structure in an unrelated protein, annexin (PDB ID: 1DK5_A) with nil segment overlap measure. Segment overlap measure is a parameter used to compare the secondary structure of two protein segments. In beta-amyloid peptide, the secondary structure of VLRDA is EEECC whereas in other unrelated protein it possesses a helical conformation (HHHHH). Similarly, 18th and 20th pentapeptides also have a completely different secondary structure in unrelated proteins which is clearly revealed from table 7.1. Since pentapeptide ‘VLRDA’ is composed of three hydrophobic (V, L and A) and two hydrophilic amino acid residues (R and D) paves the way to form a beta strand. Identical amino acid sequence fragments, which adopt different secondary structures in proteins, are termed as chameleon sequences, recent studies have revealed that chameleon sequences are significantly enriched in proteins possessing amyloidogenic sequences.
<table>
<thead>
<tr>
<th>No</th>
<th>Pattern/Secondary Structure (3M0G)</th>
<th>Identical patterns in PDB and its secondary structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>CILRD</td>
<td>IVEGA N 2COQ A</td>
</tr>
<tr>
<td></td>
<td>EECEE</td>
<td>EEEEEE</td>
</tr>
<tr>
<td>4</td>
<td>NCOILR</td>
<td>IVEGA N 2COQ A</td>
</tr>
<tr>
<td></td>
<td>EECEE</td>
<td>EEEEEE</td>
</tr>
<tr>
<td>3</td>
<td>INCVL</td>
<td>IVEGA N 2COQ A</td>
</tr>
<tr>
<td></td>
<td>EECEE</td>
<td>EEEEEE</td>
</tr>
<tr>
<td>2</td>
<td>TINGV</td>
<td>Y37 A 2COQ A</td>
</tr>
<tr>
<td></td>
<td>EECEE</td>
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<td>1</td>
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</tr>
<tr>
<td></td>
<td>EECEE</td>
<td>EEEEEE</td>
</tr>
</tbody>
</table>

Table 7.1. Results of a sub-sequence search of overlapping pentapeptides of $\beta$-amyloid in PDB sequences.
These chameleon regions have structural plasticity and involve in various biological functions. According to Chou-Fasman the amino acid residues positioned at 8-19 explicit strong alpha helix forming propensity. Crystal structure of beta-amyloid peptide (3MOQ) clearly reveals the above position as a beta strand, that the core amino acid residues in the beta-amyloid peptide have structural ambiguity. To support the results of Chou-Fasman propensities, we have also performed neural network secondary structure prediction analysis as depicted in Figure 7.1. The results of neural network secondary structure prediction are in agreement with that of Chou Fasman predictions.

**Figure 7.1.** Results of Chou-Fasman and Neural network based secondary structure prediction for (18-41)
7.3.2. Thermal unfolding simulations of β-amyloid peptide

To further dig in whether heuristically found chameleon pentapeptide is amyloidogenic or nonamyloidogenic, thermal unfolding simulations were carried out by using CNA (Constraint Network Analysis) server, which profiles that percolation and rigidity indices. CNA not only uses thermodynamics simulations considering temperature dependence of hydrophobic tethers, but also computes a set of global and local indices to predict structural weak spots/ transition points. A cluster of amino acid residues with lower the percolation index can undergo conformational dynamics. Low percolation index and rigidity index for the chameleon pentapeptide (VLDRA) as revealed by unfolding simulation studies explains is lower signifying its potential to undergo a transition as depicted in Figure 7.2.

![Percolation and Rigidity Index](image.png)

**Figure 7.2.** Thermal unfolding simulations correlating percolation and rigidity index
In order to identify the aggregation-prone regions (APR) in proteins and peptides, several computational tools have been developed using various descriptors. *viz* beta strand propensity, hydrophobicity, charges etc. We employed a profound descriptor called foldamyloid, to predict amyloidogenic cum non amyloidogenic amino acid residues in a protein. Foldamyloid program output (Figure 7.4), the profile value of amino acid residues which are amyloidogenic in nature depicting the value of chameleon pentapeptide above the threshold value. A trend line having a threshold value >21.4 signifies the value of the amino acid residues prone to aggregation and vice versa. Our thermal unfolding simulations and prediction of APRs ameliorate its potential amyloidogenic nature.

![Foldamyloid program output](image)

**Figure 7.4.** Prediction of amyloidogenic sequences in the β-amyloid peptide
7.4. Conclusion

The information to code a standard fold of a protein is present in its amino acid sequences. The conformational transitions lead to several neurodegenerative disorders in human and these transitions in a protein can be examined by looking the propensity of amino acid residues to adopt helix or a β-strand. Our detailed structural/heuristic characterization of the β-amyloid peptide from shark has made us come up with a novel chameleon pentapeptide having a potential to undergo structural transition. To check the propensity of the pentapeptide, we carried out thermal unfolding simulations and prediction of APRs. Based on the simulations, percolation index, rigidity index and APR predictions have led to the interesting findings of a pentapeptide (VLRDA) having an amyloidogenic potential. This study is first of the kind to identify chameleon pentapeptide derived from a crystallizable fragment of shark protein have the ameliorating potential for amyloidogenesis.
Conclusion of the Thesis

From cross Docking, protein-protein network analysis and detailed molecular Dynamics simulation strategies, CDK5/p25 is identified as most effective AD target which can be inhibited by flavones like Luteolin and synthetic staurosporine derivatives.

From System pharmacology approach, Mitogen activated Protein Kinase is projected with its role in AD pathology as an essential target and two phenyl sulfonyl derivatives with putative anti-MAPK activity are taken for crystallographic studies.

Based on 3D-QSAR and Pharmacophore modelling and molecular dynamics simulation studies, a staurosporine based scaffold compound has been found as an inhibitor for PKC targeting Alzheimer’s disease.

Moreover, DPP-IV is brought out as an important target for tackling type 3 diabetes as inferred from network analysis.