In silico study: effect of surfactin against amyloid β peptide was studied by using computational approaches

5.1. Introduction

Biosurfactants widely used in microbial enhanced oil recovery (MEOR), agriculture, food, cosmetics and pharmaceuticals industries. Several biosurfactants also showed antibacterial, antifungal, antiviral, anti-adhesive, and anticancer activities. As a therapeutic application, biosurfactants have many advantages such as low toxicity, high biodegradability, low irritancy, and compatibility with human skin (Kameda et al. 1974; Han et al. 2008).

Among all the classes of biosurfactant, surfactin is one of the most powerful and important lipopetide biosurfactants. Its potential applications such as inhibition of fibrin clot formation, formation of ion channels in lipid membranes (Khopade, Ren et al. 2012), antitumor activity against Ehrlich's ascite carcinoma cells (against breast cancer, colon cancer, and leukemia), antiviral activity against human immunodeficiency virus 1 (HIV-1)) (Li et al. 2005; Kim et al. 2007), inhibition of cAMP phosphodiesterase (Mulligan 2005). Surfactin kills the mycoplasmata by disrupting the membrane (Rodrigues et al. 2006).

Surfactin contains β-hydroxy fatty acid of the chain lengths 12 to 16 carbon atoms to form a cyclic lactone ring structure as shown in Figure 5.1. The amphiphilic character of surfactin is due to presence of polar amino acids (Glu-1 and Asp-5) which counterbalance the fatty acyl moiety. In aqueous phase surfactin adopts characteristic horse-saddle shaped conformation, that is probably responsible

for its broad spectrum of biological activities (Seydlová et al. 2008; Shaligram et al. 2010).

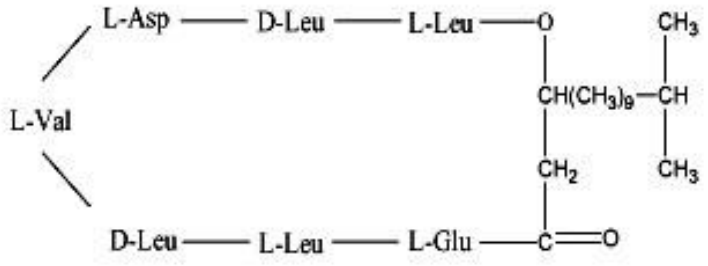


Figure 5.1. Surfactin structure.

Surfactin reported as an anti-Alzheimer' agent, its micelle formation inhibits the aggregation of amyloid beta peptide and formation of β-sheets and fibrils (Park et al. 2013). The aggregation of amyloid β-peptide (Aβ42) into fibrils is a key pathological process associated with Alzheimer's disease (Jellinger et al. 1998).

Amyloid beta is generated by the sequential cleavage of amyloid precursor protein (APP) by the two protease enzyme namely α and β secretase (Hamaguchi et al. 2006). Amyloid plaques are neurotoxic, induce stress and inflammatory response which ultimately lead to neural cell death (Jellinger et al. 1998). The major hallmark pathology for Anti-amyloidogenic Alzheimer's disease is extracellular plaque deposits of β amyloid peptides (Aβ), aggregation and deposition of Amyloid beta (Aβ) leads to neural cell death (Devarajan et al. 2014). As these amyloid plaques are neurotoxic, induce stress and inflammatory response which ultimately lead to neural cell death (Jellinger et al. 1998). Anti-amyloidogenic therapy primarily involves the reduction of Aβ production, increasing Aβ clearance, or blocking Aβ aggregation (with antibodies, peptides, natural

molecules that selectively bind and inhibit Aβ aggregate and fibril formation) (Kumar et al. 2011). Destabilization of preformed Aβ fibrils is also an interesting therapeutic intervention. A number of molecules have been reported to inhibit Aβ fibrillogenesis or to modulator of Aβ fibrillization thereby inhibiting Aβ-mediated cellular toxicity resulting from soluble amyloid oligomers or prefibrillar aggregation intermediates (Lührs et al. 2005).

Computational approach has been earlier used to study the effect of various molecules on amyloid beta and fibril. As it is reported that surfactin micelle interact with amyloid aggregates and inhibit the aggregation and deposition of amyloid beta (Aβ) (Han et al. 2008), Here our aim is to find the effect of single molecule of surfactin on amyloid beta (Aβ) through the *In silico* study, In best of our knowledge there is no *In silico* approach of study conducted to revel how single molecule of surfactin molecule interacts with amyloid beta (Aβ).

To explore this, *In silico* approach was applied to know the interaction site for single molecule of surfactin with amyloid beta 42 and amyloid fibril. The selection of surfactin is also due its environmental friendly nature, nontoxic and stable at wide range of pH and temperature as compare to chemical compounds. To reveal the common bindings of surfactin to both amyloid fibril and amyloid beta molecular docking was performed and molecular dynamics simulation studies were performed to validate the docking studies of amyloid beta and further used to analysis binding mechanism and effect of binding. This study constitutes a new frontier with a template for in vitro and in vivo experimentation in reference to new. In future this could potentially allow neuroscientists to adopt this *In silico* approach for the development of novel

surfactin based therapeutic interventions in the neuroprotection and neurotherapy of Alzheimer‘s disease.

5.2. Materials and methods

Protein preparation

3D structures of amyloid fibril Protein Data Bank (PDB) ID 2BEG (Zhang et al. 2011) and amyloid beta 42 PDB ID 1IYT (Crescenzi, Tomaselli et al. 2002) were retrieved from the PDB. 3D structures of both the protein were determined by NMR method. Amyloid fibril is pentapeptide contain N- terminal and C-terminal β-strand with a loop connecting each other. This model is perfect for the study as reported earlier, all hydrogen, including non-polar, kollman charges, and solvation parameters were added to all atoms for both the protein. After adding charges, the non-polar hydrogen’s were merged (Berendsen et al. 1995).

Ligand preparation

Surfactin structure was retrieved from pubchem (http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=443592&loc=ec_rcs) (Bolton et al. 2008) and 3D co-ordinates of surfactin were generated by the openbabel 2.3.1 software package (GhattyVenkataKrishna et al. 2013). Steepest descent algorithm and MMFF94 force filed were applied for 500 numbers of steps to optimize ligands geometry implemented in Avogadro software package(Hanwell et al. 2012).

Blind Docking

As amyloid beta and fibril is small peptide and does not have any active site or specific activity region. Blind docking approach was applied to identify possible binding regions (Balaji et al. 2013) for the surfactin on the amyloid beta and amyloid fibril.

Different software's has its own scoring function, docking score and threshold hydrogen bond criteria. Autodock is open source most cited software to predict the binding conformation for the MD simulation (Kroemer 2007; Morris et al. 2009). Molecular Docking of Surfactin was done with the Autodock 4.2 (Morris et al. 2009; Kumar et al. 2011) for generating ligand atom map autogrid was created around the both amyloid beta and fibril X 126 Y 70 and Z 80. Grid centre was placed at X = -0.619, Y=0.557, Z=9.143. Ten independent docking runs were carried out for surfactin using these parameters. The best docked position was determined by considering the total energy value of different docking poses.

Molecular dynamics

Molecular simulation was performed only on amyloid fibril and bound surfactin with the help of gromacs 4.5.5 (Lemkul et al. 2010) package with standard GROMOS96 53A6 force field (Lemkul et al. 2010). Stacey R. Gerben et al. compared five popular atomistic force fields (AMBER03, CHARMM22 + CMAP, GROMOS96 53A6, GROMOS96 54A7, and OPLS-AA) to determine which could best model the structure of Aβ. The united atom parameter sets (GROMOS96 53A6 and 54A7) may be preferable because the simulation systems will contain fewer atoms, thus making simulations faster. In this regard, trajectories conducted using OPLS-AA and GROMOS96 53A6 both converged very quickly while other force fields took longer to reach a stable state (Gerben et al. 2014).

Amyloid fibril simulation was performed to see the behaviour under physiological condition. Second simulation run was performed for the deviation from the control run in the presence of surfactin. The initial surfactin topology files were generated using the

PRODRG servers (Lindahl et al. 2001). The protein was put into space filling cubic boxes of 8nm x 8nm x 8nm and filled with single-point charge water molecules. To neutralize the system sodium ions were added. Further structures energy was minimized using steepest descent approaches. NPT and NVT canonical ensemble were used for 100 ps to equilibrate the systems. LINCS and smooth particle mesh Ewald (PME) were used for bond length constrained and long range electrostatics respectively. Molecular dynamics simulation run was conducted for 10ns. During simulation run coordinates of the protein and protein inhibitor were recorded at 2ps interval. Root mean square deviation value (RMSD) & root mean square fluctuation value (RMSF) of the main chain backbone atoms for protein and protein ligand complex were calculated. Hydrogen bonds, radiuses of gyration, solvent accessible surface area and salt bridge distance were calculated for depth analysis.

5.3. Results and Discussion

Amyloid β-protein (Aβ), appear to require essential contribution from both hydrophobic and ionic interactions during structure formation with hydrophobicity providing a large energetic contribution (Lobanov et al. 2008). Apart from providing the stabilization energy, these non-bonding interactions provide loose network structures, so that Aβ peptide can tolerate residue replacements at packing positions without losing its stability or shape. Considering these generalized rules of protein structure, it has been assumed that interaction of molecules to a site on Aβ peptide with significant specificity may inhibit amyloid fibril formation and other types of aggregation (Medina-Franco et al. 2011).

The chemical disruption of this β-sheet containing polymers was exemplified approximately two decades ago, The different chemical compounds reported previously to bind amyloid, to modulate protein aggregation and/or toxicity or screened for such activities were modelled. In this study, the surfactin molecule was used as an anti-alzheimer molecule through the molecular docking study to amyloid fibril and further study of the molecular dynamics of surfactin with amyloid fibril compound.

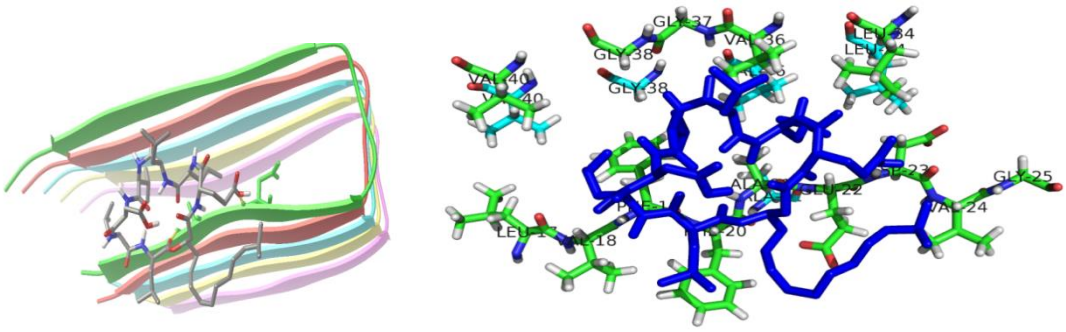
Docking of surfactin

Molecular docking of surfactin molecule on amyloid fibril revealed that the surfactin molecule interacts with A chain of amyloid fibril. Surfactin molecule head forms hydrogen bond with residues Ala 21 and Asp 23 of chain A amyloid fibril. Hydrogen bond distance for Ala 21 is 1.88 Å and for Asp 23 it was 1.80 Å. The energies of these two bonds were - 0.435 kcal/mol and -7.67 kcal/mol respectively. Docking of surfactin and amyloid beta give the total free energy of -3.28 kcal/mol.

There is no defined threshold value of free energy/ hydrogen bonds for optimum effect of a ligand/putative drug; it depends on number of factors such as size and conformation of complex, hydrogen bonds to respective amino acids, and hydrophobicity in protein-protein complex (Kitchen et al. 2004; Kroemer 2007). Even ligands showing low value of free energy with complex are also considered as drug because of their stable complex conformation and bonding of desirable targeted amino acids (Hernández-Rodríguez et al. 2015).

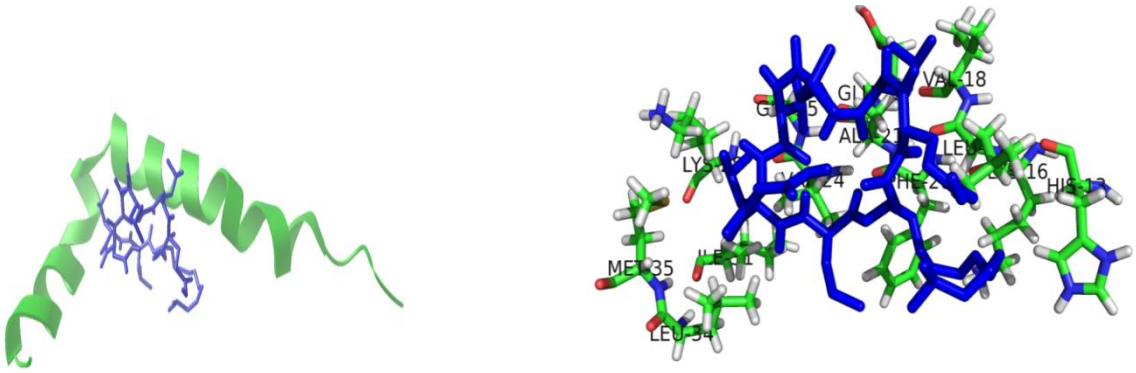
The assembly of amyloid Beta to amyloid fibers is due to the hydrophobic effect, these assemblies of amyloid fiber were stabilized by hydrophobic effect so hydrophobic

residues play an important role in amyloid fibril stabilization (Morris et al. 2009). molecular docking of surfactin to amyloid fibril and amyloid beta shows that the surfactin binds the common hydrophobic residues of amyloid fibril and amyloid beta i.e, Leu 34, Ala 21, Gly 25, Val 18, Phe 20 as shown in Figure 5.2 and 5.3. Polar residues Asp23, Lys28 and hydrophobic residues Ala21, Ile32, Leu34 and Val36 form the amphiphilic pore in Aβ peptide (Morris, Huey et al. 2009). Residue Asp23 and Lys28 also form the salt bridge which contributes the stability of Aβ peptide and also responsible for the formation of amphiphilic pore in Aβ peptide (SchuÈttelkopf et al. 2004; O'Boyle et al. 2011). Molecular docking study revealed the hydrogen bond formation with the salt bridge residue Asp 23 and hydrophobic core residue Ala21 with surfactin molecule which might lead to cause instability of the amyloid fibril.



(a) (b)

Figure 5.2 (a) Docking view of surfactin (stick) to amyloid fibril (ribbon). (b) surfactin (blue color) molecule, amino acid residue chain A (green) and chain B (cyan) with in the 5 Å area of surfactin.



(a) (b)

Figure 5.3 Docking view of (a) surfactin (blue) to amyloid beta-peptide (green), (b) surfactin (blue) to amyloid beta-peptide (green) shows the residue near 5 Å around.

Molecular dynamics simulations

To estimate the stability of docked complex and interaction effect on amyloid fibril, 10ns Molecular dynamic simulations was performed for unbound amyloid fibril and surfactin bound amyloid fibril. In both simulations, the rmsd values, rising rapidly in the first 2 ns of the simulation, protein ligand complex was stabilizes around ~6 ns afterwards quite stable during rest of simulation whereas amyloid fibril show the little deviation in RMSD *i.e.*, approximately 0.25nm in Figure 5.4. RMSD trajectory of protein ligand complex showed that the protein ligand complex is stabilizing during the simulation. i.e, surfactin amyloid fibril compound complex is stable. During the simulation run trajectory are stable after 5.6ns to 10ns, while unbound protein faced changed after 7ns whereas surfactin amyloid fibril Complex maintained its compactness. The trajectory shows that with the progress in time the complex became more compact and stable this shows strong bonding between the surfactin and amyloid fibril.

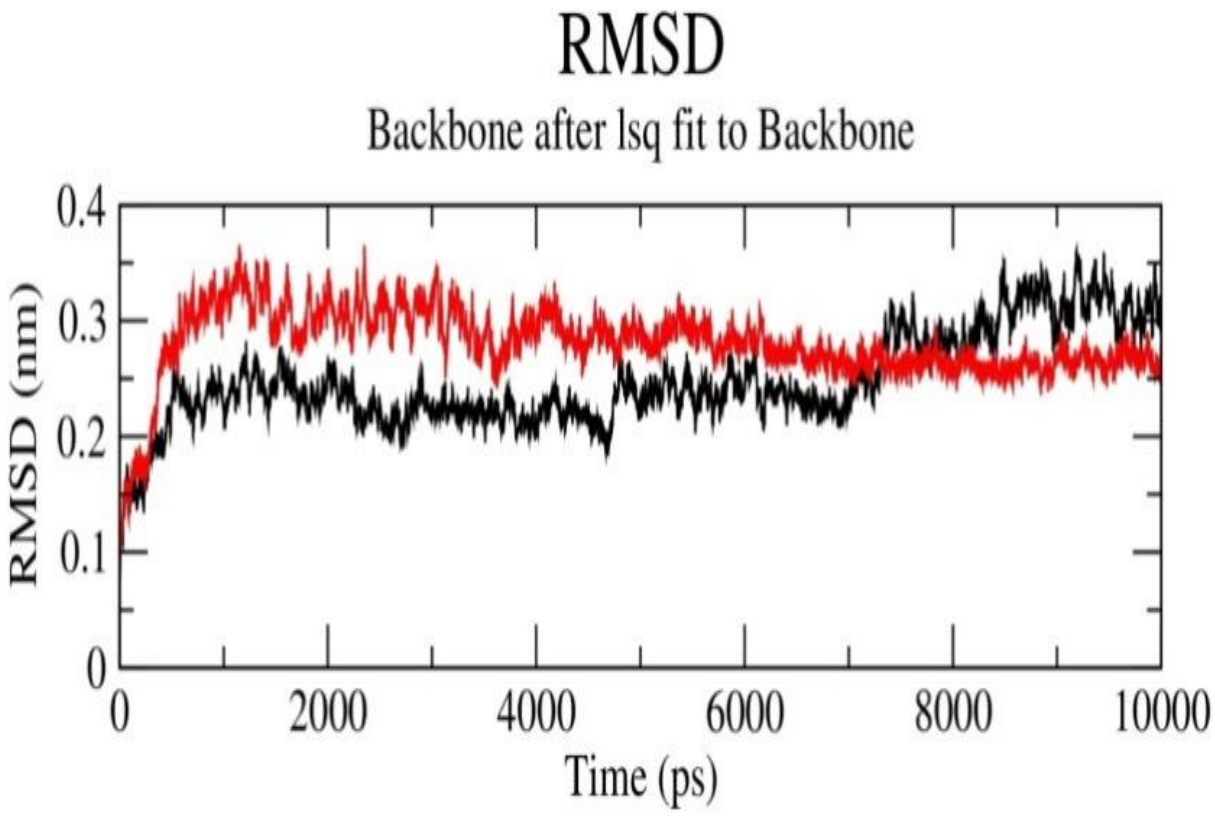


Figure 5.4 RMSD graph of amyloid fibril (black) and Surfactin + amyloid fibril complex (red).

Further the compactness of surfactin amyloid complex was measured by plotting the radius of gyration (Rg) versus time. The radius of gyration is characteristic of the compactness of protein structures (Soreghan et al. 1994). Amyloid fibril show overall stable Rg value around 1.45nm but for the surfactin amyloid complex Rg value decreases with time and stabilizes after 4ns, further decrease and then stabilize around ~1.42nm during complete simulation. It was clearly visible from Figure 5.5, that Rg value of complex was very less after 4ns comparable to unbound protein. Thus we may conclude that the surfactin amyloid complex is become more compact and stable during

the simulation, and further suggested the tight bonding between the amyloid fibril and surfactin.

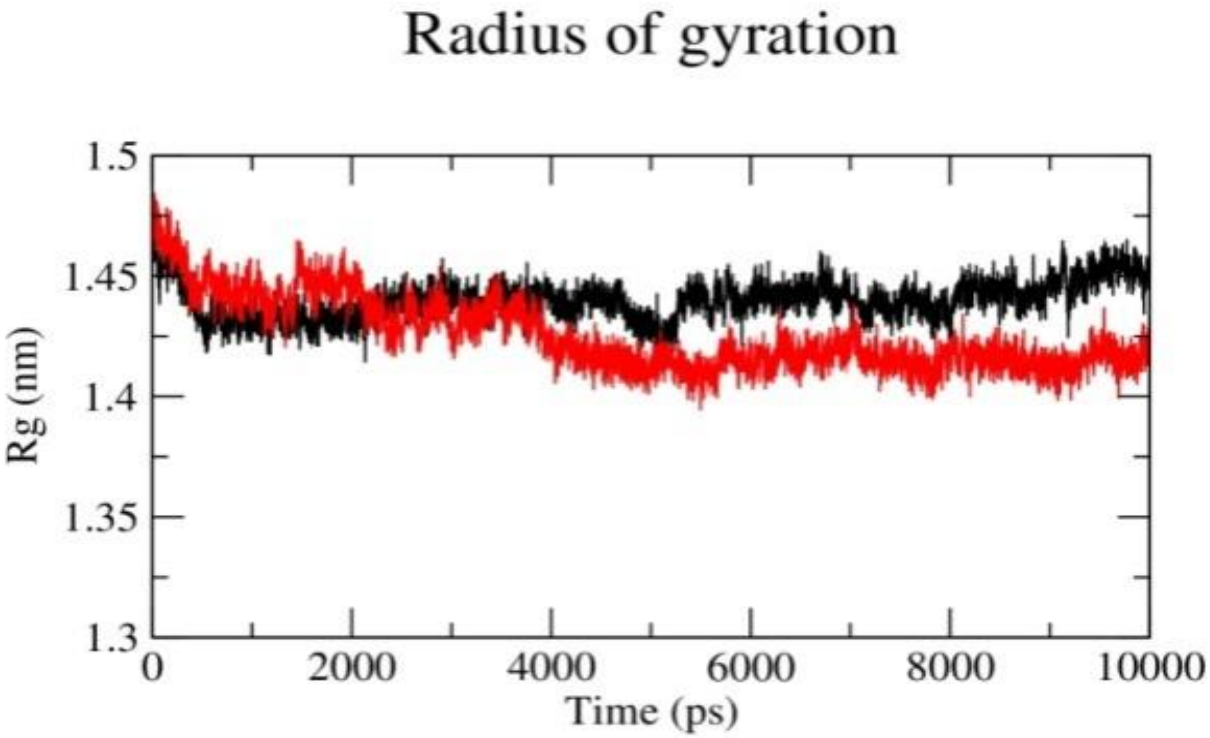


Figure 5.5. Radius of gyration graph of amyloid fibril (black) and Surfactin + amyloid fibril complex (red).

Further RMSF plot was generated (as shown in Figure 5.6) to analyse the effect of surfactin on amino acid residues of amyloid fibril. From the unbound amyloid fibril simulation, it was clear that the most flexible regions of each peptide are the C-terminus (residues 40-42) and those residues in the vicinity of the bend region (residues 25-30) that connects the two β-strands in each peptide (Soreghan et al. 1994). Terminal residue and bend region (25-30) is most flexible region (SchuÈttelkopf et al. 2004). In our simulation similar results found in case of unbound protein but in the case of bound complex this region relatively low fluctuations figure. Whereas stretch from 33 to 39 showed 3nm deviation larger the unbound complex. The fluctuation between Ala21

and Asp23 residues are less in case of docked protein. Surfactin molecule tends to stabilize these residues by forming hydrogen bond.

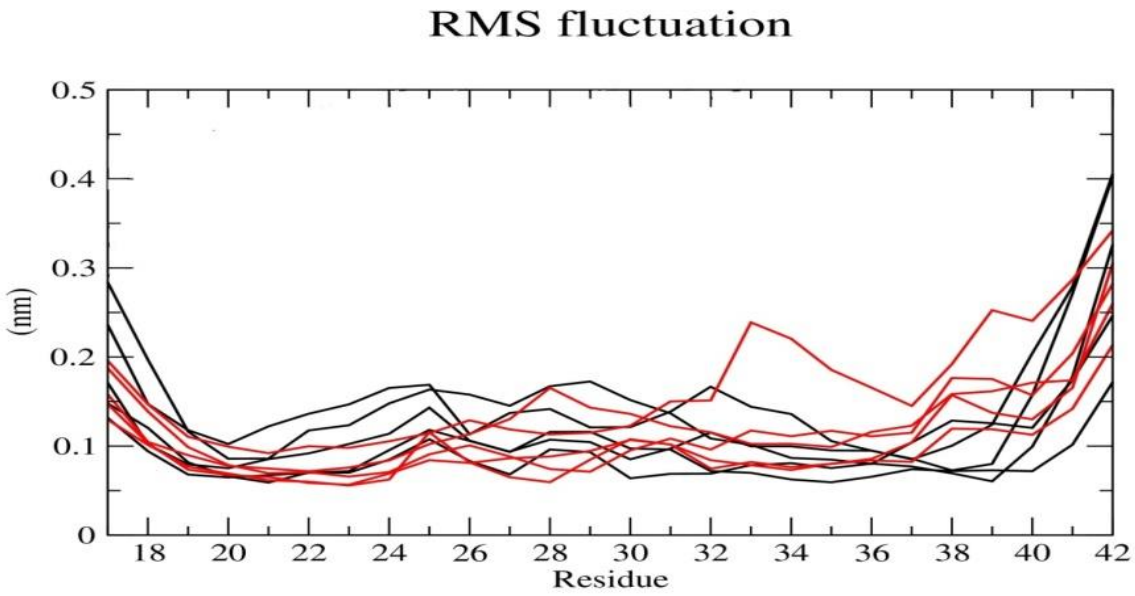


Figure 5.6. RMSF graph of amyloid fibril (black) and Surfactin + amyloid fibril complex (red).

Full entrance of surfactin into the core of the Aβ protofibril was not observed in this conformation, surfactin binds to the surface of the Aβ protofibril. It was observed that, surfactin had a tendency to deposit on the surface of the protofibril and prevent the further deposition of plaques. This study justify that there is a finite probability of surfactin entering into the hydrophobic core of the Aβ protofibril. The receptive amino acid residues involved in the formation hydrophobic core are Leu 34, Val 18, Ile31, Phe 20, Ala 21 and Gly 25. The amino acids glutamic acid and asparagine are the main polar components of surfactin that counterbalance the fatty acyl moiety and give the molecule its amphiphilic character.

Single molecule of surfactin interacted through hydrogen bonds with the Ala21 and Asp23 residues in simulation. These interactions allowed surfactin molecule to stable bind at this location. The relative agreement of the surfactin position in simulation provides strong evidence that surfactin may interact with the salt bridge, a region of the Aβ structure that is accessible from the surrounding solvent, to gain entry into the hydrophobic core and lead to destabilization. Such positioning nears the Ala21 and Asp23 which contribute for salt bridge, disrupted these ionic interactions and allowed surfactin to compete for backbone hydrogen bonds between peptides.

This work has explored the effects of deposition of surfactin on the surface of the protofibril, the potential for surfactin to penetrate into the hydrophobic core and the resulting destabilization of protofibril, and the ability of surfactin to prevent the attachment of an incoming Aβ peptide to the preformed protofibril. The principal mode of protofibril destabilization by surfactin is exerted through interactions with the Ala21 and Asp23 that have previously been proposed to be key for the stability of the mature Aβ42 fibril. The contacts formed between surfactin and these residues principally involve the hydrogen bonding groups. To see the effect of surfactin molecule on amyloid fibril structure we measured the distance between the residues Asp23 to Lys 28 which form a salt bridge for chain as shown in Figure 5.7.

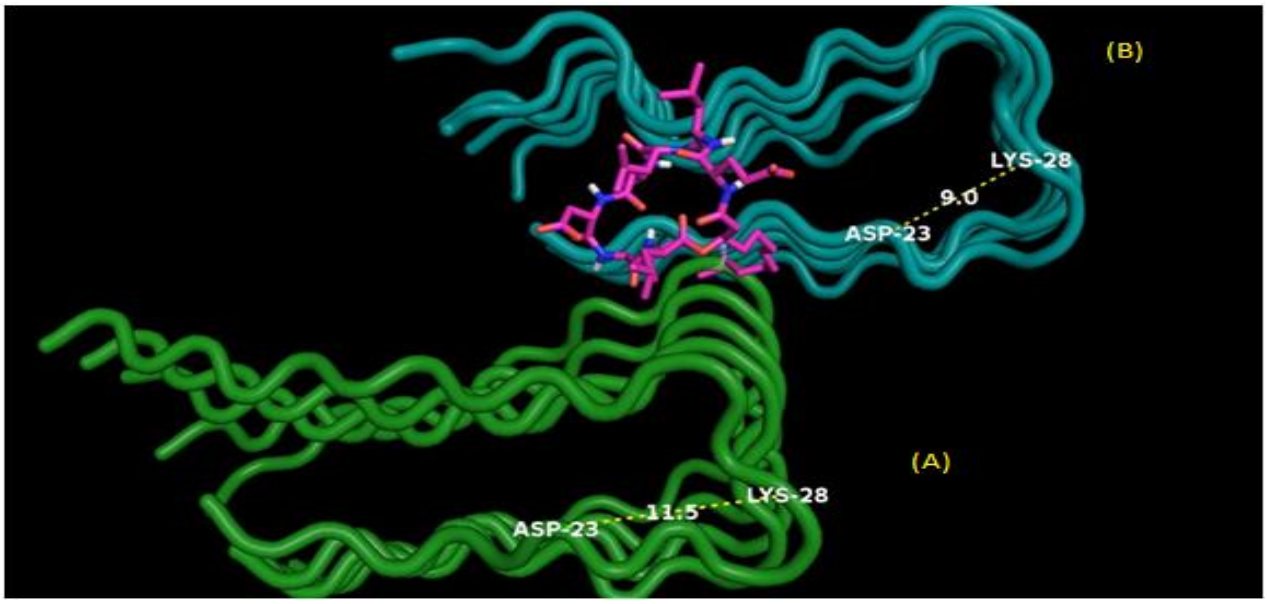


Figure 5.7. Salt bridge length of (A) amyloid fibril without surfactin molecule (11.5 Å) and (B) amyloid fibril with surfactin (9.0 Å).

Result revealed that after binding of surfactin molecule on amyloid fibril distance between the residue decreases significantly which results that each chain come close to each other. This structural change reduces the size of amphiphilic pore and leads to displacement of water molecule and destabilizes the Aβ peptide.

Solvent Accessible Surface Area

The solvent accessible surface area (SASA) is an important parameter for mapping unfolding. Additionally, the solvent accessible surface area (SASA) was analysed. A change in the SASA value represents the rearrangement of the hydrogen-bond network between amino-acid side chains and surrounding water molecules. 10000 Pico second (ps) SASA simulations was performed for unbound amyloid fibril and surfactin bound amyloid fibril and change of SASA with time is shown in Figure 5.8.

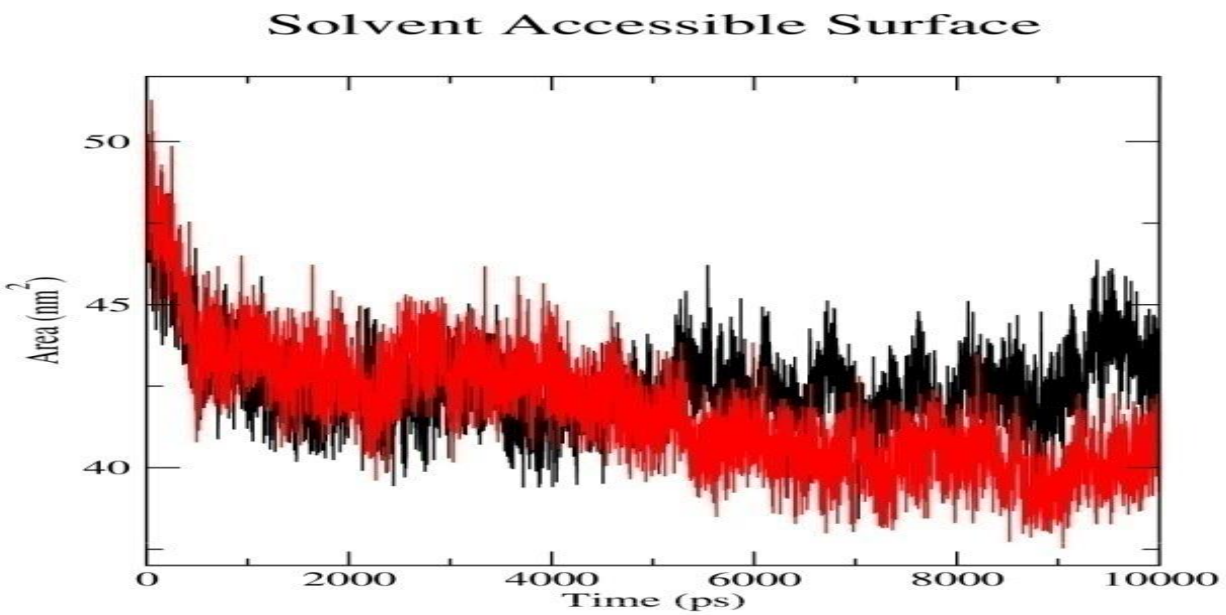


Figure 5.8. SAS graph of amyloid fibril (black) and Surfactin + amyloid fibril complex (red).

In our simulations, SASA decreases rapidly upto 2100ps then comes to platue upto 4100ps in both the case amyloid fibril and surfactin amyloid fibril. Then the decrease in area will continue for surfactin amyloid fibril complex only and after 9000ps it is showing the stable conformation. amyloid fibril does not showing the decrease in SASA plot as in case of Surfactin amyloid fibril shows during the simulation time. Decreased value of SASA plot in surfactin amyloid fibril complex structure denotes its relatively shrunken nature as compared to the amyloid fibril structure. Surfactin amyloid fibril complex and amyloid fibril structures showed similar fashion of deviation till 4500ps from the initial structure, but after this surfactin amyloid fibril complex showing decrease in SASA plot. The flexibility loss for surfactin amyloid fibril is observed in RMSD, RMSF, Rg plot which is further supported by a decrease in SASA value.

5.4. Conclusion

This work explores the effect of surfactin molecule binding onto amyloid fibril and amyloid beta. Docking studies revealed strong binding to Aβ-fibrils and amyloidal beta-42. According to docking result surfactin form stable hydrogen bond to salt bridge residue (Ala 21 and Asp 23) that is very important for the amyloid structure stability.

Molecular dynamics simulation shows the stable and compact binding of surfactin with Aβ protofibril. Surfactin binds to the surface of amyloid fibril so due to this it may inhibit the further deposition of amyloid fibril. The stability of surfactin amyloid fibril complex was observed in RMSD, RMSF, and Rg trajectory which is further supported by a SASA plot.

Soreghan et al., (1994) revels that surfactant destabilize A beta fibril due to micelles formation in solution. This study has shown that how single molecule of Surfactin (lipopeptide) interact with Ab fibril and destabilize it. To the best of our knowledge, this is novel *In silico* approach of study, which reveals the destabilization of amyloid beta (Aβ) due to single molecule of surfactin interaction. This work has explored the effects of deposition of surfactin on the surface of the protofibril, and its potential to penetrate into the hydrophobic core and amphiphilic pore to displace the water molecule which results destabilization of protofibril, and the ability of surfactin to prevent the deposition of an incoming Aβ peptide to the preformed protofibril. This *In silico* study of surfactin against the Aβ amyloid fibril responsible for Alzheimer provides information for furthering drug design for the treatment of Alzheimer's disease in the future.