A Molecular Dynamics Study on RAGE-Aβ42 Interaction and the Influence of G82S RAGE Polymorphism on Aβ Interaction

Sreeram Krishnan, Srinidhi Ravi, Sivabaakiyam Ponmalai, P. Rani*

Department of Biotechnology
PSG College of Technology
Peelamedu, Coimbatore, Tamilnadu, India
E-mails: sreeram.krishnan@yahoo.in, srinidhira@gmail.com, sivab1993@gmail.com, rani@bio.psgtech.ac.in

**Corresponding author*

Received: June 23, 2015 **Accepted: November 09, 2015**

Published: December 22, 2015

Abstract: *Interaction of amyloid peptides (Aβ) with receptor for advanced glycation end products (RAGE) elicits an inflammatory response and augments Alzheimer's disease (AD) pathology. The present study was aimed to analyse the interactions of different forms of Aβ42 peptide with ligand binding domain of normal and G82S RAGE and their possible consequences in AD pathology. The structures of RAGE ectodomain (3CJJ), monomeric forms of Aβ42 – 1IYT (apolar) and 1Z0Q (polar) and fibrillar (2BEG) were obtained from PDB. The structure of G82 and S82 RAGE was generated using SWISS MODEL. SIFT and PolyPhen analysis was performed to predict the phenotypic and functional effect of the amino acid substitution. The G82 and S82 variant structures were simulated in GROMACS and the 10 lowest energy structures were docked with different forms of Aβ42 using CLUSPRO in antibody mode. The lowest energy docked structure was further simulated for 5 ns. The structures corresponding to 0-5 ns were taken and the amino acid interactions were generated using PDBSUM. SIFT analysis indicated that G82S SNP had a tolerating effect on the structure of protein but polyphen predicted a probable damaging effect. Highest binding score was obtained with 2BEG docked with both G82 RAGE (–375.84 ± 7.425 Kcal/mol) and G82S variant (–391.09 ± 13.391 Kcal/mol) indicating that the fibrillar form showed better interaction. Compared to G82 RAGE, the S82 variant showed better interaction to all three forms of Aβ42. The results of study indicate that RAGE interacted better with fibrillar form of Aβ42 peptide and G82S mutation enhanced the binding affinity of RAGE towards amyloid peptides leading to enhanced inflammatory response.*

Keywords: *RAGE, Aβ42 peptide, Alzheimer's disease, G82S Polymorphism, Molecular dynamics, Protein-peptide docking.*

Introduction

Alzheimer's disease (AD) is the most common form of dementia [7]. AD is characterised by the presence of amyloid (Aβ) plaques and neurofibrillary tangles (NFT) leading to changes in the neuronal homeostasis and architecture by altering cerebral blood flow inducing oxidative and nitrosylative stress by the formation of free radicals and generation of advanced glycation end products (AGEs) [6]. The AGEs play a major role in ageing and is reported to be co-localised along with the plaques and NFTs, implying their response in AD pathology [34, 35]. AGEs elicit an inflammatory role via the NF-κB pathway by activation following interaction with its receptor RAGE (Receptor for advanced glycation end products) [39].

Aβ peptides in brain are the main component of senile plaques. Apart from the brain, the source for Aβ peptides in plasma are skeletal muscles, platelets and vascular walls [23, 38]. RAGE acts as a receptor for Aβ peptides and transport them into the brain across the blood brain barrier [5, 14] and augment the disease by increasing the inflammatory condition. Binding of Aβ to RAGE also activate the amyloid precursor protein (APP) cleaving enzyme, BACE1, thereby increasing Aβ production [8]. In addition, inhibition of long term potentiation (LTP) in hippocampal neurons by Aβ, also depend on RAGE [32]. Thus RAGE is an important candidate in AD pathology.

Understanding the specifics of interaction between RAGE and its ligand would serve as prelude to development drugs that block such interaction. Koch et al. [21] identified the amino acid residues of RAGE involved in binding with AGEs and S100 peptide. Chaney et al. [6] used a dimeric model of RAGE and docked Aβ42. However, concrete information on the amino acids involved in interaction with Aβ peptide is lacking.

In the ligand binding region of RAGE several SNPs such as A28V (rs17846804), R48Q (rs35030981), R77C (rs116828224), G82S rs114177847) and R114Q (rs17846806) have been reported. G82S polymorphism in the ligand binding domain is shown to upregulate inflammatory response in arthritis [20]. While Li et al. [25] and Daborg et al. [12] have reported an association between G82S RAGE polymorphism and AD, influence of this mutation on interaction with Aβ peptides is not reported. Aβ peptides have also been reported to adopt different conformation in different solvents [17]. Hence, the present study was undertaken to evaluate the interaction of RAGE towards different conformational variants of Aβ peptides and also to evaluate the influence of G82S polymorphism in the ligand binding domain of RAGE on its interaction with Aβ using a molecular dynamic approach.

Materials and methods

RAGE and Aβ42 structures

The monomeric crystal structure of RAGE ectodomain (3CJJ) consisting of amino acids 23-240 which includes the ligand binding domain and C1 constant domain was used for the present study [21]. Sequence of 3CJJ was retrieved from PDB and G82S mutation was introduced. Using 3CJJ as reference, homology modeling was done using SWISS-MODEL [2]. Three forms of Aβ42 namely 1IYT, a monomeric structure in apolar environment [10], 1Z0Q, a solution structure in a polar environment [37] and 2BEG, a fibrillar form of Aβ42 [28] were docked with G82 and S82 forms of RAGE.

Prediction of deleterious effect of G82S SNP

SIFT and Polyphen-2 servers were used to predict the effect of G82S SNP on the structure and function of the protein. Sorting Intolerant from Tolerant (SIFT) is a homology based tool that predicts if an amino acid change alters the phenotypic nature of the protein. SIFT predictions are based on the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences, collected through PSI-BLAST. Scores ranging from 0-0.05 are considered to be deleterious whereas scores ranging from 0.05-1 indicate that the amino acid change is tolerated or neutral [22, 30, 31]. The query was submitted in the form of dbSNP. PolyPhen (Phenotyping Polymorphisms) software version 2.0.9 predicts the functional effects of an amino acid change. Two pairs of datasets are used for prediction viz. HumDiv and HumVar. HumDiv complies all damaging alleles, affecting the molecular function of the protein that cause human Mendelian diseases and compares their differences between human proteins and their related homologous proteins present in the Uniprot database, which are assumed to be non-damaging. HumVar provides a score by

accounting for all human disease-causing mutations (MAF > 1%) from UniProtKB, together with common human non synonymous SNPs (treated as non-damaging) without annotated involvement in disease. The amino acid change is classified as benign, possibly damaging and probably damaging based on the false positive rate (FPR) thresholds [1]. PolyPhen analysis was done with the uniprot ID:Q15109 along with SNP position and amino acid variation.

Simulation, docking and amino acid interaction

The wild type (G82) and homology modeled S82 RAGE ectodomain structures were subjected to molecular dynamics simulations using GROMACS Version 4.0 [3]. The structures were solvated with SPC water model in GROMOS96 53A6 force field in a box size of 2 cubic cm and energy minimized using conjugate-gradient algorithm (CG). The minimized system was subjected to a 100ps simulation to temperature and pressure variation (nvt, npt) and final simulation was carried out in an isothermal-isobaric ensemble of 300K and 1 atm pressure for 5 ns. The resultant trajectory was saved and 10 lowest energy structures were chosen for docking with different forms of Aβ42 peptides.

The ten lowest free energy structures of both G82 and S82 RAGE were docked with monomeric and fibrillar forms of Aβ42 using Cluspro 2.0 docking program. Since the ectodomain of RAGE is similar to the Ig binding region, the protein was docked with Aβ42 in a modified antibody mode [4]. The structure with lowest free energy was utilized for calculating the binding score. The binding scores of the 10 structures were statistically analysed by one way ANOVA followed by Tukey's post-hoc comparison using SPSS 10.1 platform.

To analyse the stability of the docked conformation, further simulations of the lowest energy docked structure of G82 and S82 forms were carried out for 5ns by following the same protocol. The resultant trajectory was saved and structures corresponding to 0, 1, 2, 3, 4 and 5 ns were analysed for change in the amino acid interaction via PDBSUM.

Results and discussion

Prediction of deleterious effect of G82S SNP using SIFT and PolyPhen

The RAGE sequence was submitted to SIFT and PolyPhen servers to analyse the phenotypic and functional effects of the amino acid mutation on the protein. SIFT score of 0.67 showed that G82S had a tolerating effect on the phenotype but PolyPhen predicted that the amino acid change probably had a damaging effect on the function of the protein (Table 1). SIFT incorporates position-specific information by using sequence alignment and is intended specifically for predicting whether an amino acid substitution affects the phenotype [30, 31]. SIFT predicted that G82S amino acid change was observed to have a tolerable effect on the RAGE structure. PolyPhen-2 predicted the functional significance of an amino acid replacement by naïve Bayes classifier. The SNP is predicted to be probably damaging, possibly damaging and benign based on the False positive rate (FPR) values. The amino acid change is predicted to be probably damaging if its probability scores is at or below the first FPR (lower) value and SNPs whose probability scores fall below the second FPR value are predicted to be possibly damaging. Mutations with FPRs above the second FPR value are classified as benign [1]. PolyPhen predicted the G82S amino acid change as probably damaging. The probability score associated with G82S amino acid change with estimated false positive rates were below the first FPR value; hence it was predicted to have a damaging effect on the function of the protein.

Table 1. SIFT and PolyPhen analysis of G82S polymorphism in RAGE

SNP	Amino acid change	Tool	Prediction	Score
rs114177847	G 82 S	SIFT	Tolerated	0.67
		PolyPhen	Probably Damaging	1.00

Conformational stability of G82 and S82 RAGE structures during MD simulation

G82S mutation was created in the RAGE ectodomain and the G82 and S82 structures were subjected to molecular dynamics for 5 ns. Molecular dynamics of RAGE structures showed a stable conformation throughout simulation as suggested by RMSD (Fig. 1) and Ramachandran plots (Fig. 2). Ramachandran plot indicated that the mutated amino acid stayed in the allowed region of the plot throughout the simulation, signifying structural stability of the variant. RMSD scores of the selected structures revealed minor change in conformation when compared to the lowest energy structure of G82 RAGE (Table 2).

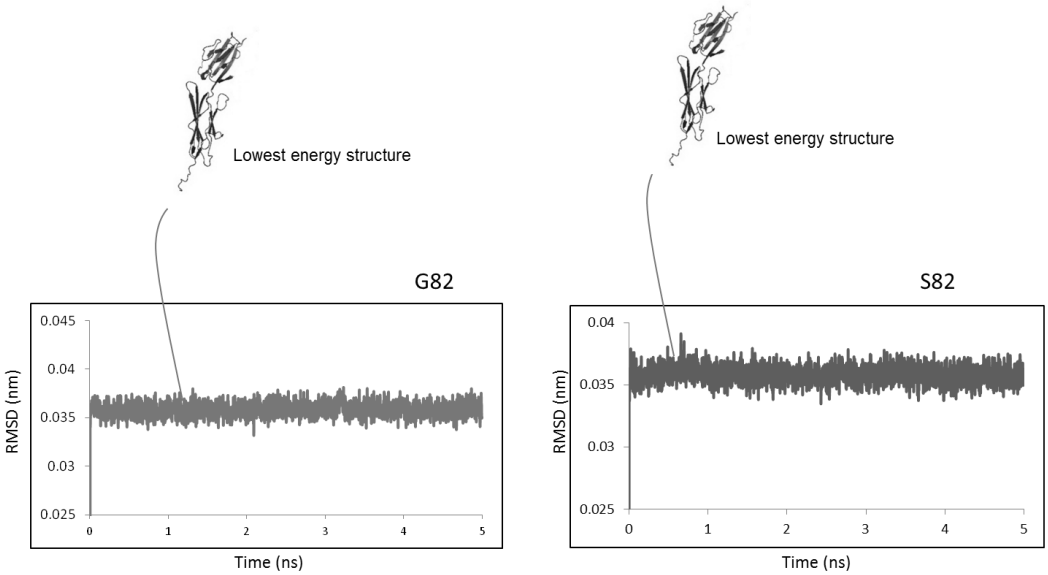

Fig. 1 RMSD plot of G82 and S82 RAGE structures during MD simulation

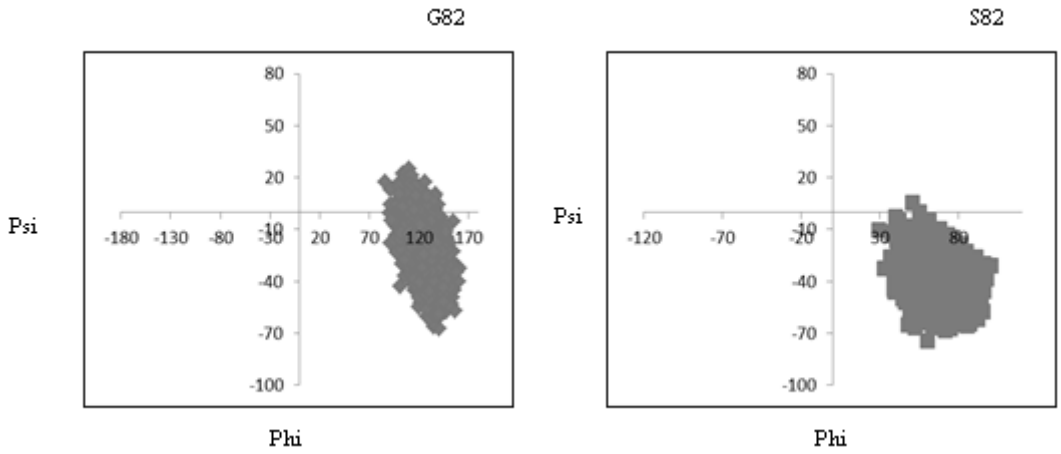

Fig. 2 Ramachandran plot of the G82 and S82 amino acids of RAGE during MD simulation

Table 2. Structural variation (RMSD) of G82 and S82 RAGE

	RMSD, (Å)	
	Carbon alpha (CA)	Backbone
G82 RAGE	0.287 ± 0.012	0.330 ± 0.007
S82 RAGE	0.528 ± 0.009	0.585 ± 008

Values are expressed as mean ± standard deviation

RAGE binds with fibrillar form of Aß more effectively

G82 RAGE, when docked with monomeric (apolar:1IYT, polar:1Z0Q) or fibrillar (2BEG) forms of Aβ42 (Fig. 3) gave a binding score of –312.78 ± 8.085 Kcal/mol, –353.72 ± 7.018 Kcal/mol, and –375.84 ± 7.425 Kcal/mol respectively (Table 3), which suggests that RAGE bound to fibrillar form more effectively than the other forms.

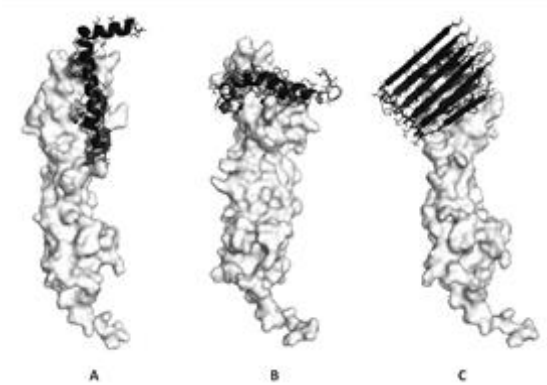

Fig. 3 Docking conformation of RAGE with Aβ42. RAGE docked with the three different conformational forms of Aβ42 (black): 1IYT (A), 1Z0Q (B) and 2BEG (C).

Table 3. Docking of RAGE variants with Aβ42 peptides

	Binding scores, (Kcal/mol)		
	1IYT	1Z0Q	2BEG
G82 RAGE	–312.38 ± 6.477	–353.72 ± 7.018^{*a}	–375.84 ± 7.425^{*a,b}
S82 RAGE	–318.78 ± 8.085	–359.54 ± 10.485^{*a}	–391.09 ± 13.391^{*a,b}

Values are expressed as mean ± standard deviation

*a – significantly different from 1IYT scores, $p < 0.001$

*a,b – significantly different from 1IYT and 1Z0Q scores, $p < 0.001$

Simulation of the docked structure was performed to validate its stability. Structures corresponding to 0-5 ns, at 1 ns interval were retrieved and their amino acid interaction was analysed using PDBSUM. The conformation of the structures were stable thorough out the simulation (Fig. 4) with stable amino acid interaction (Table 4).

It has been indicated that a dimeric form of RAGE is involved in ligand binding and further signal transduction. Chaney et al. [6] used a dimeric model of RAGE and docked Aβ42 and results indicated that K52, K62 and R98 of one subunit bound to D1, E3 and D7 of Aβ42 and interactions were the same for the other subunit too. Hence a monomeric form of RAGE was used for docking with Aβ42 in the present study and the results indicated that Q24, K37, K39, K43, K52, R104, N105, R98, Q100, E108, and K110 of RAGE were mainly involved in binding with Aβ42 peptides. Deane et al. [15] had indicated that several positively charged patches on the V-domain of RAGE, including R29, K37, K39, K43, K44, R48, K52, R98,

R104, K107, K110, R114 and R116 may interact with negatively charged regions on Aβ (Residues 17-23: LVFFAED) along with the hydrophobic cavity close to the C1 terminal consisting of I30, P87, A88, I91 and Y11. Similarly Koch et al. [21] reported that the RAGE amino acids, R48, R98, and patch around R104, K39, V89, E94, F97, A101, N105, E108 and T109 were involved in binding with AGEs and S100 peptide. Similar interactions were observed in the present study. Though the majority of the amino acids involved in interaction with Aβ42 peptide are resides in the variable domain, interactions were also observed between amino acids in the C1 domain (L214, R216) and Aβ42 peptides. Matsumoto et al. [29] have indicated that other domains too play a role in ligand binding, although the variable domain was the binding site for AGEs. Similarly, S100B bound to both the variable and C1 domains while S100A6 bound to C1 and C2 domains [24]. Dattilo et al [13] have suggested that the variable and C1 domains are not independent, but rather form an integrated structural unit for ligand recognition.

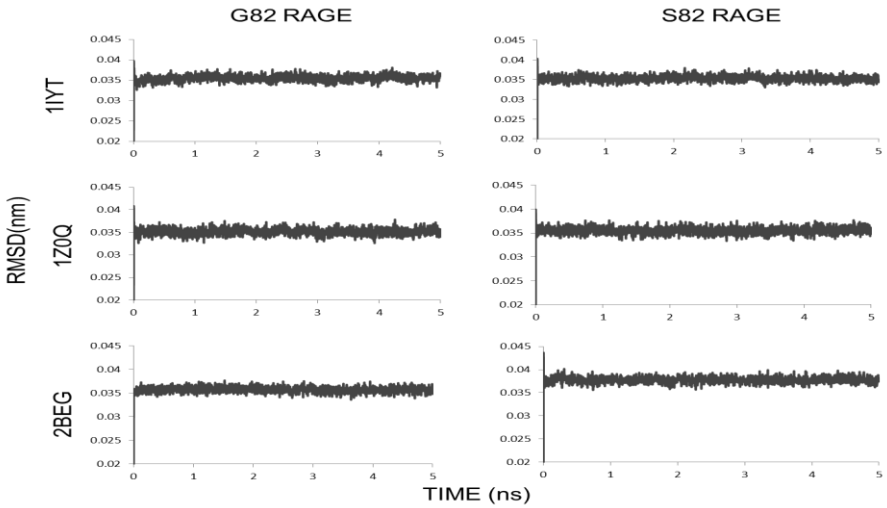

Fig. 4 RMSD plot of G82 and S82 RAGE structures docked with three forms of Aβ42 peptides

Among the three forms of Aβ42 peptide, the fibrillar form showed better amino acid interaction with RAGE compared to the other forms (Table 4) as suggested by the binding scores. Difference in amino acid interactions was observed between the three forms. Salt bridges were observed between R216-D1 for 1IYT and between K39-D7, K110-E22 and R98-E22 for 1Z0Q while no salt bridges were observed for 2BEG. K39 of G82 form had a stable hydrogen bond with E22 of 2BEG where as K39 interacted with E11 of 1IYT indicating a change in interaction with change in the form of Aβ42 peptide. The number of hydrogen bonds and salt bridges between G82 RAGE and 2BEG were lesser compared to the other forms of Aβ42 peptide. This could be attributed to the fact that the amino acids in the individual sheets of the fibril would be interacting with each other to maintain the stability of the fibril and only fewer amino acids were involved in G82 RAGE interaction.

Luhrs et al. [28] indicated that the probable intersheet side chain interactions were formed between residues F19-G38, A21-V36 and a salt bridge between D23-K28 in the fibril. Analysis of the intersheet bonding of 2BEG via PDBSUM (data not shown) revealed that most of the amino acids of a chain in 2BEG had an interaction with the neighboring chains of the fibril. Though the number of hydrogen bonds and salt bridges between G82 RAGE and 2BEG were observed to be lesser, the free energy contributed by the intra chain hydrophobic interactions was higher thereby increasing the binding score. Moreover, the cumulative effect

of the individual β sheet (totally five sheets) interactions in the fibril could contribute to the increase in the binding score between G82 RAGE and 2BEG.

Table 4. Amino acid interactions of G82 RAGE and S82 variant with Aβ peptides

	Common amino acid interaction for G82 and S82 forms	**Specific for G82 RAGE**	**Specific for S82 RAGE**
1IYT			
Hydrogen bonds	K39-**E11**, K43-**E22**, R116-**E3**, R216-**D1**	Q24-**E11**, K37-**D7**, K37-**S8**, K43-**D23**, Y113-**E11**, R216-**D1**	N25-**D7**, K37-**Q15**, N112-**Y10**
Salt bridges	NIL	R216-**D1**	K43-**E22**
Non bonded contacts	Q24-**Y10**, Q24-**E11**, Q24-**D7**, N25-**F4**, I26-**D7**, T27-**F4**, K37-**E11**, K37-**D7**, K39-**H14**, K39-**Y10**, K39-**E11**, G40-**V18**, A41-**V18**, P42-**E22**, K43-**E22**, R116-**E3**, R216-**D1**	N25-**E3**, K37-**S8**, K43-**F19**, K43-**D23**, Y113-**E11**, R116-**F4**, L214-**F4**	M22-**Y10**, N25-**D7**, K37-**Q15**, N103-**A21**, N103-**V18**, N112-**Y10**
1Z0Q			
Hydrogen bonds	K37-**E3**, K52-**N27**, R98-**E22**, Q100-**N27**	**Y10**9-**E11**, N103-**E11**	R98-**S26**, K110-**Q15**, S111-**E22**
Salt bridges	R98-**E22**	K39-**D7**, K110-**E22**	
Non bonded contacts	K37-**D1**, K37-**E3**, K39-**D7**, K52-**N27**, W61-**L34**, W61-**A30**, R98-**E22**, R98-**S26**, Q100-**N27**, T109-**Q15**, K110-**Q15**, K110-**D23**	C38-**E3**, G40-**E11**, A41-**F4**, K43-**F4**, K52-**S26**, N81-**E3**, **S8**3-**E3**, R98-**G25**, Q100-**D23**, N103-**E11**, N103-**F4**, E108-**Q15**, T109-**E11**	M22-**H4**, M22-**A21**, Q24-**D7**, W61-**S26**, R98-**A21**, C99-**E22**, S111-**E22**, N112-**E22**
2BEG			
Hydrogen bonds	K43-**A21**, K43-**E22** (A Chain), K37-**E22** (C Chain), K39-**E22** (D Chain)	K43-**E22** (B Chain)	K37-**E22** (B Chain), K37-**E22**, Q24-**E22** (D Chain)
Salt bridges	NIL	NIL	NIL
Non bonded contacts	K43-**E22** (A Chain), K37-**E22**, G40-**F20** (C Chain), Q24-**E22**, Q24-**F20** (D Chain), I26-**E22**, K37-**E22** (E Chain)	K43-**E22** (B Chain)	A41-**F20**, K43-**F19**, K43-**A21**, N103-**F20**, R104-**V18** (A Chain), K37-**E22**, G40-**F20**, A41-**F20**, N103-**V18** (B Chain), Q24-**E22** (D Chain), N25-**E22** (E Chain)

*Amino acids for Aβ42 are depicted in bold.

The binding score of G82 RAGE towards 1Z0Q was higher when compared to 1IYT. The number of salt bridges and non-bonded contacts were higher for 1Z0Q when compared to 1IYT (Table 4) which indicates that G82 RAGE interact better with 1Z0Q than 1IYT. Moreover, 1IYT is modeled in an apolar solvent and is reported to have an α-helical structure [10] while 1Z0Q is modeled in a polar environment [37]. Hence the solvent might also play a role in determining the binding interaction. RAGE, being a transmembrane receptor, has it ligand binding domain exposed to the hydrophilic environment. Since 1Z0Q is modeled in a polar environment, the hydrophilic residue of the peptides is more exposed when compared to 1IYT. Hence 1Z0Q is likely to show a better interaction when compared to 1IYT as suggested by the scores (Table 3). Moreover, Tomaselli et al. [37] also reported that simulation of 1Z0Q in a polar environment, changes the conformation of the peptide from α-helical to a β sheet. β sheet conformation is also found in the fibrillar form which may have a better affinity to RAGE than a α helix conformation.

G82S mutation enhances the binding interaction of RAGE towards Aβ42 peptides

The interaction of S82 variant towards Aβ42 peptides followed a similar trend as in G82 RAGE with 2BEG showing highest binding score and 1IYT having the least score (Table 3). Compared to G82 RAGE, the binding energies of the S82 variant docked to Aβ peptides was lower by ~16 Kcal/mol for the fibrillar (2BEG) forms and by ~6 Kcal/mol for monomeric (1IYT and 1Z0Q) forms of Aβ42 indicating a possible increase in binding affinity of the variant to all forms of Aβ42 (Table 3). Change in amino acid interactions were observed for S82 variant with 1IYT, 1Z0Q and 2BEG (Table 4, Fig. 5). In Fig. 5 the amino acids are indicated in different colors and the hydrogen bonds between the amino acids are indicated in a dotted yellow line. Hydrogen bonds were observed between N25-E3 and K37-D7 in G82 RAGE while N25 and K37 of the S82 variant bonded with D7 and Q15 respectively indicating a change in interaction as a result of the amino acid change. A salt bridge was observed between K43 and E22 in the S82 variant, while a salt bridge was observed in the C1 domain (R216-D1) for G82 RAGE. Non-bonded interactions were observed between N81 and S83 of G82 RAGE with E3 of 1Z0Q. But introduction of a serine residue at the 82nd position leads to the dissolution of the same in the S82 variant (Table 4).

Reports indicate that G82S polymorphism amplifies the inflammatory response via NF-κB pathway [20], and the increased inflammatory response could be attributed to increased N-linked glycosylation of N81 residue [33]. The conformational change caused due to amino acid change (G82S) could be the possible reason behind enhanced glycosylation at N81. Srikrishna et al. [36] reported that glycosylation takes place at N25 and N81 and their types were complex and hybrid/high mannose respectively. Yet, reports on the degree of glycosylation and individual residue type is lacking and hence molecular dynamics and docking studies could not be carried out with glycosylated form of RAGE.

Association of G82S polymorphism with AD: enhanced binding affinity to Aβ42 and altered clearance mechanism

A soluble form of RAGE (sRAGE) is generated by alternative splicing of RAGE mRNA and by proteolytic cleavage of full lengh RAGE (flRAGE) [9, 16]. sRAGE differs from flRAGE by its lack of signaling property due to the absence of trans-membrane domain, but contains the ligand binding domain, thereby acting as a decoy receptor and competes with flRAGE for ligands. The ectodomain of RAGE (3CJJ) used in the study represents both sRAGE and flRAGE. The increase in binding affinity towards sRAGE would result in effective clearance of the Aβ peptide. But, a decreased expression of sRAGE [18, 26] and increased expression of

flRAGE [7, 27] is reported in AD. Daborg et al. [12] also reported that 82S carriers have lower sRAGE levels than 82G carriers and flRAGE could be engaged in a positive feedback mechanism, further reducing sRAGE levels. In addition, 82S allele increased the BACE1 activity, impairing the clearance of Aβ42 peptide and promoting their aggregation [19]. Hence, G82S mutation might favour the binding of Aβ42 to flRAGE and augment the disease condition by promoting aggregation of peptide.

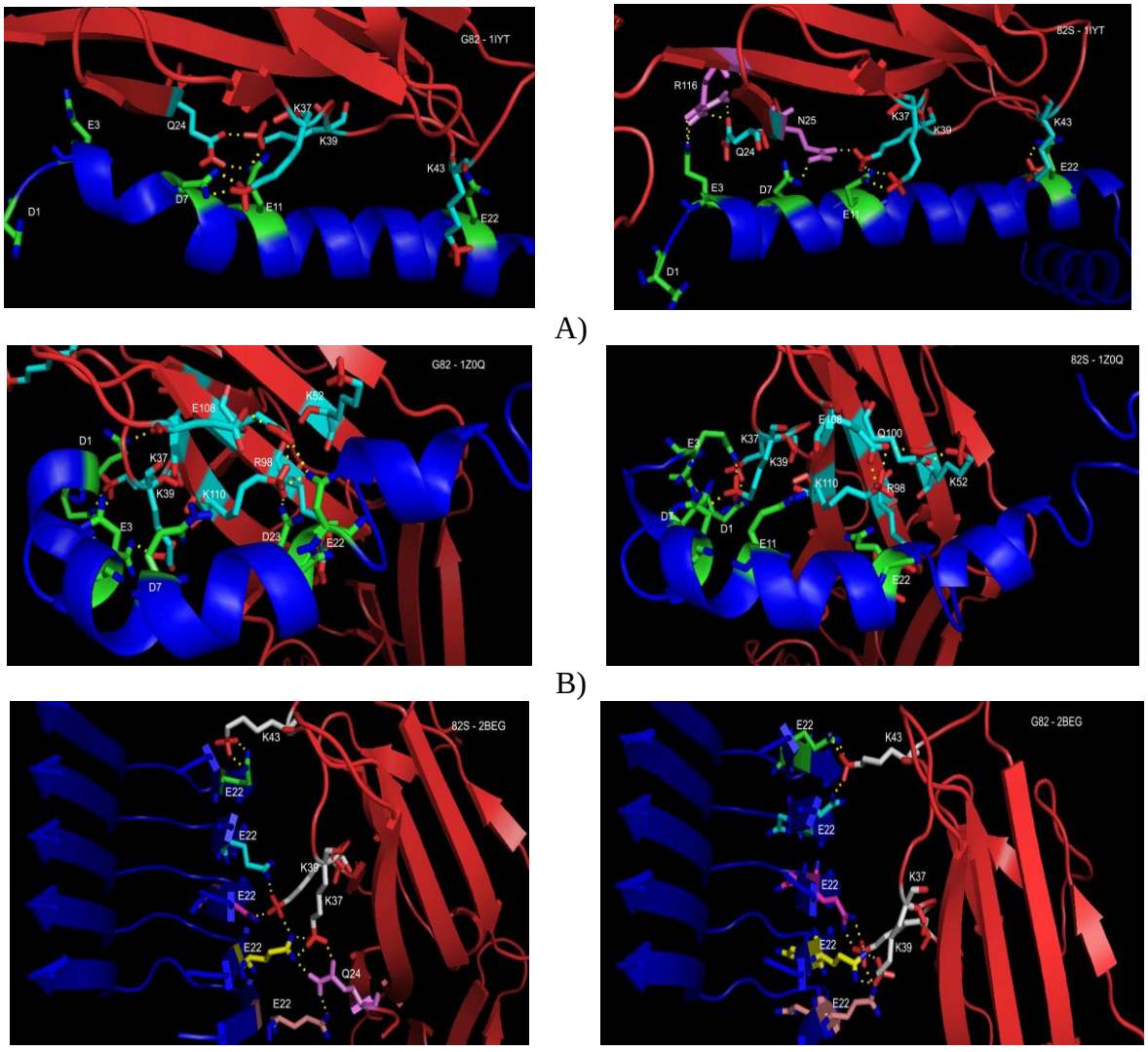

Fig. 5 Amino acid interaction between RAGE (red) and three different conformational forms of Aβ42 peptides (blue): 1IYT (A), 1Z0Q (B) and 2BEG (C).

Conclusion

The present molecular dynamics study revealed that the amino acids Q24, K37, K39, K43, K52, R104, N105, R98, Q100, E108, and K110 in the ligand binding domain of RAGE were mainly involved in binding with Aβ42 peptides and the fibril form of Aβ42 peptide bound more efficiently with RAGE. G82S mutation in RAGE enhanced its the binding affinity towards Aβ42 peptides, leading to enhanced inflammatory response and thereby augmenting AD pathology. The results of the present study also provide insights about the amino acid interactions between RAGE and Aβ42 peptide.

Acknowledgements

The authors thank Dr. C. Sabarinath, Dr. S. Vidyalakshmi, Dr. V.Ramamurthy, Staff and Research scholars, Department of Biotechnology, PSG College of Technology for their encouragement.

References

1. Adzhubei I. A., S. Schmidt, L. Peshkin, V. E. Ramensky, A. Gerasimova, P. Bork, A. S. Kondrashov, S. R. Sunyaev (2010). A Method and Server for Predicting Damaging Missense Mutations, Nature Methods, 7(4), 248-249.
2. Arnold K., L. Bordoli, J. Kopp, T. Schwede (2006). The SWISS-MODEL Workspace: A Web-based Environment for Protein Structure Homology Modeling, Bioinformatics, 22, 195-201.
3. Berendsen H. J. C., D. Van der Spoel, R. van Drunen (1995). GROMACS: A Message-passing Parallel Molecular Dynamics Implementation, Computer Physics Communications, 91, 43-56.
4. Brenke R., D. R. Hall, G. Y. Chuang, S. R. Comeau, T. Bohnuud, D. Beglov, O. Schueler-Furman, S. Vajda, D. Kozakov (2012). Application of Asymmetric Statistical Potentials to Antibody-protein Docking, Bioinformatics, 28(20), 2608-2614.
5. Cai Z., N. Liu, C. Wang, B. Qin, Y. Zhou, M. Xiao, L. Chang, L. J. Yan, B. Zhao (2015). Role of RAGE in Alzheimer's Disease, Cell and Molecular Neurobiology, doi: 10.1007/s10571-015-0233-3.
6. Chaney M. O., W. B. Stine, T. A. Kokjohn, Y-M. Kuo, C. Esh, A. Rahman, D. C. Luehrs, A. M. Schmidt, D. Stern, S. D. Yan, A. E. Roher (2005). RAGE and Amyloid Beta Interactions: Atomic Force Microscopy and Molecular Modeling, Biochimica et Biophysica Acta, 1741(1-2), 199-205.
7. Choi B. R., W. H. Cho, J. Kim, H. J. Lee, C. Chung, W. K. Jeon, J. S. Han (2014). Increased Expression of the Receptor for Advanced Glycation End Products in Neurons and Astrocytes in a Triple Transgenic Mouse Model of Alzheimer's Disease, Experimental and Molecular Medicine, doi: 10.1038/emm.2013.147.
8. Cho H. J., S. M. Son, S. M. Jin, H. S. Hong, D. H. Shin, S. J. Kim, K. Huh, I. Mook-Jung (2009). RAGE Regulates BACE1 and Aβ Generation via NFAT1 Activation in Alzheimer's Disease Animal Model, FASEB Journal, 23, 2639-2649.
9. Chuah Y. K., R. Basir, H. Talib, T. H. Tie, N. Nordin (2013). Receptor for Advanced Glycation End Products and Its Involvement in Inflammatory Diseases, International Journal of Inflammation, 2013, 1-15.
10. Crescenzi O., S. Tomaselli, R. Guerrini, S. Salvadori, A. M. D'Ursi, P. A. Temussi, D. Picone (2002). Solution Structure of the Alzheimer Amyloid Beta-peptide (1-42) in an Apolar Microenvironment. Similarity with a Virus Fusion Domain, European Journal of Biochemistry, 269, 5642-5648.
11. Cummings J. L. (2004). Alzheimer's Disease, New England Journal of Medicine, 351, 56-67.
12. Daborg J., M. Otter, A. Sjölander, S. Nilsson, L. Minthon, D. R. Gustafson, I. Skoog, K. Blennow, H. Zetterberg (2010). Association of the RAGE G82S Polymorphism with Alzheimer's Disease, Journal of Neural Transmission, 117, 861-867.
13. Dattilo B. M., G. Fritz, E. Leclerc, C. W. Kooi, C. W. Heizmann, W. J. Chazin (2007). The Extracellular Region of the Receptor for Advanced Glycation End Products is Composed of Two Independent Structural Units, Biochemistry, 46(23), 6957-6970.
14. Deane R., R. D. Bell, A. Sagare, B. V. Zlokovic (2009). Clearance of Amyloid-β Peptide Across the Blood-brain Barrier: Implication for Therapies in Alzheimer's Disease, CNS & Neurological Disorders – Drug Targets, 8, 16-30.

15. Deane R. J. (2012). Is RAGE Still a Therapeutic Target for Alzheimer's Disease?, Future Medicinal Chemistry, 4(7), 915-925.
16. Ding Q., J. N. Keller (2005). Splice Variants of the Receptor for Advanced Glycosylation End Products (RAGE) in Human Brain, Neuroscience Letters, 373, 67-72.
17. D'Ursi A. M., M. R. Armenante, R. Guerrini, S. Salvadori, G. Sorrentino, D. Picone (2004). Solution Structure of Amyloid β-Peptide (25-35) in Different Media, Journal of Medicinal Chemistry, 47(17), 4231-4238.
18. Emanuele E., A. D'Angelo, C. Tomaino, G. Binetti, R. Ghidoni, P. Politi, L. Bernardi, R. Maletta, A. C. Bruni, D. Geroldi (2005). Circulating Levels of Soluble Receptor for Advanced Glycation End Products in Alzheimer Disease and Vascular Dementia, Achieves of Neurology, 62, 1734-1736.
19. Ho L., W. Qin, P. N. Pompl, Z. Xiang, J. Wang, Z. Zhao, Y. Peng, G. Cambareri, A. Rocher, C. V. Mobbs, P. R. Hof, G. M. Pasinetti (2004). Diet-induced Insulin Resistance Promotes Amyloidosis in a Transgenic Mouse Model of Alzheimer's Disease, FASEB Journal, 18(7), 902-904.
20. Hofmann M. A., S. Drury, B. I. Hudson, M. R. Gleason, W. Qu, Y. Lu, E. Lalla, S. Chitnis, J. Monteiro, M. H. Stickland, L. G. Bucciarelli, B. Moser, G. Moxley, S. Itescu, P. J. Grant, P. K. Gregersen, D. M. Stern, A. M. Schmidt (2002). RAGE and Arthritis: The G82S Polymorphism Amplifies the Inflammatory Response, Genes & Immunity, 3, 123-135.
21. Koch M., S. Chitayat, B. M. Dattilo, A. Schiefner, W. J. Chazin, G. Fritz (2010). Structural Basis for Ligand Recognition and Activation of RAGE, Structure, 18, 1342-1352.
22. Kumar P., S. Henikoff, P. C. Ng (2009). Predicting the Effects of Coding Non-synonymous Variants on Protein Function Using the SIFT Algorithm, Nature Protocols, 4(8), 1073-1081.
23. Kuo Y. M., T. A. Kokjohn, M. D. Watson, A. S. Woods, R. J. Cotter, L. I. Sue, W. M. Kalback, M. R. Emmerling, T. G. Beach, A. E. Roher (2000). Elevated Abeta42 in Skeletal Muscle of Alzheimer Disease Patients Suggests Peripheral Alterations of AbetaPP Metabolism, American Journal of Pathology, 156(3), 797-805.
24. Leclerc E., G. Fritz, M. Weibel, C. W. Heizmann, A. Galichet (2007). S100B and S100A6 Differentially Modulate Cell Survival by Interacting with Distinct RAGE (Receptor for Advanced Glycation End Products) Immunoglobulin Domains, Journal of Biological Chemistry, 282(43), 31317-31331.
25. Li K., D. Dai, B. Zhao, L. Yao, S. Yao, B. Wang, Z. Yang (2010). Association between the RAGE G82S Polymorphism and Alzheimer's Disease, Journal of Neural Transmission, 117, 97-104.
26. Liang F., J. Jia, S. Wang, W. Qin, G. Liu (2013). Decreased Plasma Levels of Soluble Low Density Lipoprotein Receptor-related Protein-1 (sLRP) and the Soluble Form of the Receptor for Advanced Glycation End Products (sRAGE) in the Clinical Diagnosis of Alzheimer's Disease, Journal of Clinical Neuroscience, 20(3), 357-361.
27. Lue L. F., D. G. Walker, L. Brachova, T. G. Beach, J. Rogers, A. M. Schmidt, D. M. Stern, S. D. Yan (2001). Involvement of Microglial Receptor for Advanced Glycation End Products (RAGE) in Alzheimer's Disease: Identification of a Cellular Activation Mechanism, Experimental Neurology, 171, 29-45.
28. Luhrs T., C. Ritter, M. Adrian, D. Riek-Loher, B. Bohrmann, H. Dobeli, D. Schubert, R. Riek (2005). 3D Structure of Alzheimer's Amyloid-beta(1-42) Fibrils, Proceedings of National Academy of Sciences USA, 102, 17342-17347.
29. Matsumoto S., T. Yoshida, H. Murata, S. Harada, N. Fujita, S. Nakamura, Y. Yamamoto, T. Watanabe, H. Yonekura, H. Yamamoto, T. Ohkubo, Y. Kobayashi (2008). Solution

Structure of the Variable-type Domain of the Receptor for Advanced Glycation End Products: New Insight into AGE-RAGE Interaction, Biochemistry, 47, 12299-12311.
30. Ng P. C., S. Henikoff (2003). SIFT: Predicting Amino Acid Changes that Affect Protein Function, Nucleic acid Research, 31(13), 3812-3814
31. Ng P. C., S. Henikoff (2006). Predicting the Effects of Amino Acid Substitutions on Protein Function, Annual Review of Genomics and Human Genetics, 7, 61-80.
32. Origlia N., S. Capsoni, A. Cattaneo, F. Fang, O. Arancio, S. D. Yan, L. Domenici (2009). Abeta-dependent Inhibition of LTP in Different Intracortical Circuits of the Visual Cortex: The Role of RAGE, Journal of Alzheimer's Disease, 17, 59-68.
33. Park S. J., T. Kleffmann, P. A. Hessian (2011). The G82S Polymorphism Promotes Glycosylation of the Receptor for Advanced Glycation End Products (RAGE) at Asparagine 81: Comparison of Wild-type Rage with the G82S Polymorphic Variant, Journal of Biological Chemistry, 286, 21384-21392.
34. Sasaki N., S. Toki, H. Chowei, T. Saito, N. Nakano, Y. Hayashi, M. Takeuchi, Z. Makita (2001). Immunohistochemical Distribution of the Receptor for Advanced Glycation End Products in Neurons and Astrocytes in Alzheimer's Disease, Brain Research, 888(2), 256-262.
35. Smith M. A., S. Taneda, P. L. Richey, S. Miyata, S. D. Yan, D. Stern, L. M. Sayre, V. M. Monnier, G. Perry (1994). Advanced Maillard Reaction End Products are Associated with Alzheimer Disease Pathology, Proceedings of National Academy of Sciences USA, 91(12), 5710-5714.
36. Srikrishna G., J. Nayak, B. Weigle, A. Temme, D. Foell, L. Hazelwood, A. Olsson, N. Volkmann, D. Hanein, H. H. Freeze (2010). Carboxylated N-glycans on RAGE Promote S100A12 Binding and Signaling, Journal of Cellular Biochemistry, 110, 45-59.
37. Tomaselli S., V. Esposito, P. Vangone, N. A. van Nuland, A. M. Bonvin, R. Guerrini, T. Tancredi, P. A. Temussi, D. Picone (2006). The Alpha-to-beta Conformational Transition of Alzheimer's Abeta-(1-42) Peptide in Aqueous Media is Reversible: A Step by Step Conformational Analysis Suggests the Location of Beta Conformation Seeding, ChemBioChem, 7, 257-267.
38. Van Nostrand W. E., J. P. Melchor (2001). Disruption of Pathologic Amyloid Beta-protein Fibril Assembly on the Surface of Cultured Human Cerebrovascular Smooth Muscle Cells, Amyloid, 8(1), 20-27.
39. Yan S. D., X. Chen, J. Fu, M. Chen, H. Zhu, A. Roher, T. Slattery, L. Zhao, M. Nagashima, J. Morser, A. Migheli, P. Nawroth, D. Stern, A. M. Schmidt (1996). RAGE and Amyloid-beta Peptide Neurotoxicity in Alzheimer's Disease, Nature, 382(6593), 685-691.

Sreeram Krishnan, Ph.D. Student
E-mail: sreeram.krishnan@yahoo.in

Sreeram Krishnan is a Ph.D. student in the Department of Biotechnology, PSG College of Technology (Anna University), Tamilnadu, India. He is a recipient of National Doctoral Fellowship from All India Council for Technical Education, Government of India. His research focuses on evaluating the role of selenium and selenoproteins in blood and CSF, as potential markers for early diagnosis of dementia, especially Alzhiemer's disease. He is also studying the clearance of amyloid peptides by the inflammatory receptors like RAGE and how the clearance is affected by the presence of SNPs in the receptor and is association with Alzheimer's pathology.

Srinidhi Ravi, B.Sc.
E-mail: srinidhira@gmail.com

Srinidhi Ravi is a Masters student of Biotechnology in the Department of Biotechnology, PSG College of Technology, Tamilnadu India. As a part of a student exchange program, she did a part of her M.Tech. course at the University of Luxembourg. She received her Bachelors degree in Biotechnology from Anna University, Chennai.

Sivabaakiyam Ponmalai
E-mail: sivab1993@gmail.com

Sivabaakiyam Ponmalai is a Bachelors student of Biotechnology in the Department of Biotechnology, PSG College of Technology, Tamilnadu, India. She is currently pursuing her Masters at Anna University, Chennai.

Prof. P. Rani, Ph.D.
E-mail: rani@bio.psgtech.ac.in

Dr. P. Rani is a Professor in the Department of Biotechnology, PSG College of Technology, Coimbatore, India. She obtained her Ph.D. from Indian Institute of Technology, Madras. She had gained research experience from reputed research institutes like Indian Institute of Technology, Chennai, and Indian Institute of Science, Bangalore. Her current research interest are in analyzing the expression of selenoproteins in body fluids like blood and CSF in neurodegenerative disorders especially in Alzheimer's dementia (AD) and trying to correlate their expression with the extent of neuronal damage. Other research related to AD includes:

- Development of glycogen synthase kinase (GSK-3β) inhibitor for neurodegenerative disorders;
- Influence of polymorphism in RAGE gene on the clearance of amyloid peptides;
- Development of GPS/GSM based locator device for dementia patient tracking.