Journal of the Neurological Sciences 405 (2019) 116425

Contents lists available at ScienceDirect

Journal of the Neurological Sciences

journal homepage: www.elsevier.com/locate/jns

Rational design of linear tripeptides against the aggregation of human mutant SOD1 protein causing amyotrophic lateral sclerosis

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ARTICLE INFO

Keywords:
ALS
SOD1
Peptides
DMD
FEL
Mutation

ABSTRACT

Formation of protein aggregation is considered a hallmark feature of various neurological diseases. Amyotrophic lateral sclerosis is one such devastating neurodegenerative disorder characterized by mutation in Cu/Zn superoxide dismutase protein (SOD1). In our study, we contemplated the most aggregated and pathogenic mutant A4V in a viewpoint of finding a therapeutic regime by inhibiting the formation of the aggregates with the aid of tripeptides since new perspectives in the field of drug design in the current era are being focused on peptide-based drugs. Reports from the experimental study have stipulated that the SOD1 derived peptide, "LSGDHCII-GRTLVVHEKADD" was found to have the inhibitory activity against aggregated SOD1 protein. Moreover, it was determined that the hexapeptide, "LSGDHC" was the key factor in inhibiting the aggregates of SOD1. Accordingly, we utilized the computerized algorithms and programs on determining the binding efficiency and inhibitory activity of hexapeptide on mutant SOD1. Following that, we incorporated a cutting-edge methodology with the use of molecular docking, affinity predictions, alanine scanning, steered molecular dynamics (SMD) and discrete molecular dynamics (DMD) in designing the de novo tripeptides, which could act against the aggregated mutant SOD1 protein. Upon examining the results from the various conformational studies, we identified that CGH had an enhanced binding affinity and inhibitory activity against the aggregated mutant SOD1 protein than other tripeptides and hexapeptide. Thus, our study could be a lead for state-of-the-art design in peptide-based drugs for doctoring the cureless ALS disorder.

1. Introduction

Protein aggregation is associated with various neurodegenerative disorders that in turn accumulate into amyloids and inhibit the neuronal function, thereby directing towards neurotoxicity. Reports over the preceding two eras have unveiled that the formation of oligomers during the aggregation process worsens disease progression by wielding neurotoxicity. Studies with different hypotheses suggested that these toxic oligomers mature to fibrils, which are found to be causative cytotoxic agents in augmenting the disease pathogenicity. Findings from varying structural reports disclosed that the proteins forming such toxic oligomers were found to have rich antiparallel beta sheets propensity, a key hallmark feature in neurodegenerative disordered proteins [1–4].

Amyotrophic lateral sclerosis (ALS) is envisaged as a prominent neurodegenerative disorder characterized by the denervation of upper and lower motor neurons in brain and spinal cord, thus urging towards the early onset of disease progression [5]. No therapeutics treatment for the ALS exists to date, except, riluzole and radicava, which aid barely in slowing down the disease progression [6,7]. Studies from the earlier genomics findings suggested that familial form of ALS accounts for approximately 12% of cases, while the remaining are the sporadic form [8]. Prospects for better understanding and clinical interference began with an encounter of familial and sporadic ALS cases that are caused by mutations in Cu/Zn superoxide dismutase 1 (SOD1) gene [9,10]. SOD1 is a highly conserved homodimeric metalloproteinase that scavenges the superoxide radicals [11]. Reports stated that misfolded or/and aggregated form of SOD1 proteins is a trademark feature that shows up even before symptoms, are diagnosed [12]. So far, questions on the systematic action behind the toxicity of aggregate formation, destabilization and misfolding of mutant SOD1 remain unanswered [13]. Formation of SOD1 aggregates in both, the familial and sporadic form is being considered a hallmark feature of ALS disease and thereby, draw key attention to all the avid researchers in finding a clue towards the ailment of the disease. Numerous hypothesis has been propositioned for the aggregation of mutant SOD1 [14,15]. The primary circumstance for aggregation is owed to the early detachment of SOD1 homodimer that

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https://doi.org/10.1016/j.jns.2019.116425
Received 15 February 2019; Received in revised form 11 July 2019; Accepted 9 August 2019
Available online 10 August 2019
0022-510X/

eventually influences towards the increased neurotoxicity [16,17]. In the contemporary situation, the use of small molecules and varying compounds from natural and other organic sources were utilized in inhibiting the formation of protein aggregation in SOD1 protein. Several computational and experimental studies from assorted groups have specified the use of small molecules, which might efficiently stabilize the SOD1 protein and thereby encumber the formation of protein aggregates [18,19] [20–23]. Findings from distinct research groups have insinuated that substitution of Ser at 111th position of Cys in mutant forms of SOD1 hindered the progression of protein aggregates. Moreover, the pathogenicity of the disease was also found to be reduced on inducing the mutation, C111S, on SOD1 protein in its mutant forms (H46R, A4V, G37R, G93A, and I113T) [24–26]. Lately, T2D (phosphomimetic mutation) identified by Dokholyan group suggested that the mutation (T2D) thermodynamically stabilizes and protects from the aggregates formed in A4V SOD1 mutant variant [27]. Herein, we postulated a state-of-the-art methodology with a newfangled aspect in designing the tripeptides against the formation of toxic aggregates by mutant SOD1 protein. Primarily, we intended to prefer the most deleterious, disease-causing and aggregating SOD1 mutant A4V as a test standard for our present study. From the extensive clinical reports, we could conjecture that A4V mutant is exceedingly diagnosed in patients who have familial ALS. Moreover, the rate of disease progression and severity of the disease leading towards the death were comparatively higher than all other mutant forms of SOD1 in patients [28–30]. Regarding the chemistry, the substitution of single amino acid, Val at 4th position of Ala could lead to the steric interference with distorted geometry, upon the substitution of CH_3 with the isopropyl group (C_3H_7) [31,32,34]. Besides, reports from existing experimental and computational studies have portrayed the formation of aggregates due to A4V mutation in SOD1 protein [35–37]. Furthermore, various research groups also worked on the different small molecules inhibitors to impede the formation of aggregates in A4V SOD1 mutant, using *in-silico* and in-vitro approaches [39]. From the aforementioned perceptions on the aggregation of mutant SOD1 (A4V), our study is a venture to cogently establish the use of novel tripeptides, which might act as an inhibitor against the development of toxic aggregates by mutant SOD1 protein.

Several literature studies have insinuated that the synthetic peptide derivates could act as a beta-sheet breaker, thus inhibiting the formation of amyloid aggregates in distinct aggregated proteins [40,41]. Moreover, the synthetic peptides are found to have crucial importance in preventing the formation of hydrogen bonds, which are considered to be a key component of the beta-sheet structure. Besides, the use of peptides in inhibiting the aggregates have shown promising results in various disorders such as Alzheimer's, prion, and polyglutamic diseases [42–46]. Therefore, the design of de novo peptide-based inhibitors could be promising rather than the chemically formulated compounds. Further advancement on use of peptide inhibitors as a drug candidate is more obsequious with medical ailments, due to the factors, such as high specificity, low toxicity, mode of action and ease of crossing the blood-brain barrier (BBB) [46,47]. Hence, we take into the account of the advantages above and the inhibitory effect of de novo peptides strategy in designing the tripeptides from SOD1 derived peptides that are earlier suggested to have inhibitory activity against the aggregated SOD1 protein [48]. The cutting-edge methodology proposed in our present study aid in delivering the tripeptide that could unveil greater inhibitory activity relative to SOD1 derived peptide, overcoming the disadvantage of lengthy peptide and minimal inhibitory activity against the formation of toxic aggregates of mutant SOD1.

2. Computational details

2.1. Protein structural information

Initial coordinates of mutant SOD1 protein (1UXM (A)) were obtained from PDB [49]. Mutant structure was energy minimized, using GROMACS v5.0.4 program [50]. The system was solvated using SPCE water molecules within a cubic box of size 1.0 nm. Na + ions were used to neutralize the charge of the system. Subsequently, the structure was energy minimized using steepest descent algorithm.

2.2. Protein peptide docking

Structural determination of the protein-peptide complex is vital in elucidating the mechanism behind the biological processes [51]. Consequently, we employed HPEPDOCK program for performing the protein-peptide docking studies. HPEPDOCK program uses blind protein–peptide docking over a hierarchical algorithm. The program uses MODPEP program in generating the ensemble of conformation rather than the use of lengthy simulation to obtain proper peptide conformations. Moreover, the program was found to have a high success rate in protein-peptide docking as compared to other programs.

2.3. Conformer generation

Further, the conformational ensemble of mutant-peptide complexes was generated through tCONCOORD [52] program. The program builds the geometrical constraints using the interactions present within the given structure. Accordingly, the predefined constraints entrenched in program aids in construction of structure iteratively by revising the coordinates, till the structure satisfy the geometrical constraints, thus delivering new conformations. Thereby, we created a group of 1000 mutant-peptide conformations independently and the binding affinities were computed, using PRODIGY program [53].

2.4. Alanine scanning for peptides

Erstwhile to alteration in binding affinity calculations, the peptide was subjected towards alanine scanning, using FoldX program, whereby every residue in peptides was mutated to alanine. The energy differences were computed for each mutation [54,55]. The obtained peptides were further optimized with YASARA program and utilized for further calculations.

2.5. Torsional angle and stability prediction of peptides

Consequently, we analyzed the effect of change in the amino acid of peptides using CUPSAT program [56], which predicts the stability upon the substitution of every amino acids with amino acid atom potential and torsional angle distribution.

2.6. Steered molecular dynamics (SMD)

SMD was performed on mutant-peptide complexes using YASARA program with AMBER03 force field [57]. The systems were solvated with water molecules of 0.997 g/ml solvent density at a constant temperature of 298 K. NaCl with 0.9% concentration was used for neutralizing the system. Long-range coulomb forces [58] were integrated with PBC. The continual steering acceleration of 2000 picometers/psˆ2 was used to steer the peptides from mutant-peptide complex, independently. The center mass of mutant SOD1 was kept constant while pulling peptide in a defined direction. SMD ended when the peptide gets unbound from mutant, with a distance of 0.4 nm, therefore ensuring that the peptide dissociated from the complexes, completely. The snapshots of the simulation were saved at an interval of every 10 ps.

2.7. Discrete molecular dynamics (DMD)

DMD is a distinctive molecular dynamic simulation, which uses the swift processing of event-driven molecular dynamics with the potential function for calculating interactions. Medusa force field was used for

Table 1
Key interactions of hexapeptide, LGH and CGH tripeptides with mutant SOD1.

Peptide-mutant SOD1	Hydrogen bond interactions with distance in Å	Hydrophobic interactions
LSGDHC (Hexapeptide)	Asn19 (3.08) and Ser98 (2.68)	Lys3, Val4, Val5, Phe20, Lys30, Trp32, Ile99, Glu100 and Ala152
LGH	Lys3 (2.70) and Glu21 (2.60)	Val4, Asn19, Phe20, Lys30, Trp32 and Gln153
CGH	Gln33 (3.05) and Ile99 (2.99)	Lys30, Val31, Trp32, Asp96, Val97 and Ser98

performing the dynamic calculations. Parameterized Medusa force field is designed for varied protein sequence that determines the protein fold family and explores the structural perturbation related to mutations [59,60]. Consequently, united-atom model was used for the representations. Besides, the system was solvated using the Lazaridis-Karplus implicit solvation [61]. Hydrogen bonds interactions were modeled using the Reaction-like algorithm [62]. The charge-charge interactions were modeled using the Debye-Hu¨ckel approximation, with Debye length setting to 10 Å. The simulations were carried out with periodic boundary conditions. The temperature was maintained using Andersen's thermostat 300 K [63]. Consequently, the snapshots were secured for every 100-timeunits (tu) for mutant and mutant-peptide complex. The time units in DMD simulations refer to the unit of time [T] that is determined by units of mass [M], length [L], and energy [E], which are Dalton (1.66×10^{-24} g), angstrom (10^{-10} m), and kcal/mol (6.9×10^{-22} J), respectively. The overall simulation time was of 50 ns corresponding to the classical MD. DMD is more efficient in providing improved sampling above MD allowing microsecond simulations on a normal personal computer. Moreover, DMD is a tool that is highly compatible for the study of aberrant folding intermediates, protein aggregation and ab initio protein folding appropriate to protein misfolding diseases such as Alzheimer's disease and ALS.

2.8. Geometrical analysis

Trajectories obtained from DMD simulation for mutant and mutant-peptide complexes were geometrically evaluated, using g_rmsf (conformational flexibility) and g_gyrate (protein gyration/Rg) from GROMACS tools and the statistical analysis were performed, using STATPLUS program.

2.9. Free energy landscape

Free energy landscape of protein was acquired using conformational sampling method, which provides the near-native structural conformation. Herein, we used DMD to sample the conformations of mutant and mutant peptide complex, correspondingly. To obtain the free energy landscape, we utilized two essential components such as conformational deviation (*p1*) and protein gyration (*p2*) as the reaction coordinates. The energy landscape was computed with these two components using the equation,

$$\Delta G(p1, p2) = -K_g T \ln p(p1, p2)$$

where ΔG is the Gibbs free energy of state, k_B is the Boltzmann constant, T is the temperature of simulation [64].

3. Result and discussion

Designing the peptides for inhibiting the protein aggregates was found to be an extremely thought-provoking process in the field of peptidomimetics. In our study, we distinctly proposed a cutting-edge methodology that urged towards the finding of two newly designed tripeptides (LGH and CGH) from SOD1 derived hexapeptide, "LSGDHC," which was found to have the utmost efficacy in inhibiting the aggregate formation in mutant SOD1 protein by factually acting as a beta-sheet breaker.

3.1. Influence of hexapeptide with mutant SOD1- Study 1

Initially, we retrieved the SOD1 derived hexapeptide, "LSGDHC" and performed the geometrical optimization, using semi-empirical Hamiltonian AM1 present in MOPAC package. The optimized structure of mutant SOD1 and the hexapeptide were docked, using HEXPEP program. Results predicted by the program interpreted that the peptide binds with mutant SOD1 with a binding affinity of −108.78 kcal/mol. The binding site regions of the hexapeptide with mutant SOD1 were found to be aggregation-prone region of SOD1 protein as reported in earlier studies. Moreover, the binding site reported in our study is similar to the earlier reports from experimental and computational studies. Therefore, we salvaged the binding site residues and its key interactions with mutant SOD1 (Table 1). With the results from the Table 1, we could determine that Asn19 and Ser98 revealed a strong hydrogen bond interaction with His and Leu amino acid of hexapeptide with a distance of 3.08 and 2.68 Å, respectively. Further, Lys3, Val4, Val5, Phe20, Lys30, Trp32, Ile99, Glu100 and Ala152 showed a significant hydrophobic interaction with hexapeptide. Reports from the previous experimental and theoretical studies suggested that Lys30, Trp32, Ile99, Glu100 are considered to be critical, which upon capping could aid in inhibiting the formation of aggregates in various mutant forms of SOD1. Thereby, suggesting that the results from our study were in correlation with the experimental reports. Further, we performed the SMD study on hexapeptide-mutant SOD1, where the results suggested that the timescale of 60 ps was required to pull off the hexapeptide to a distance of 25 Å from mutant SOD1. Thus, the results were directly proportional to the docking study, thereby substantiating its interactive binding affinity of hexapeptide with mutant SOD1. So far, with the results from docking and SMD study, we could only insinuate an outline on the action of a binding factor with mutant protein. However, the evidence concerning the efficacy of inhibitory action was not vibrant in providing a suggestive conclusion. Hence, we endeavored to provide an elucidative report on the inhibitory effect of hexapeptide on mutant SOD1 protein over the conformational preferences by utilizing the DMD program. Accordingly, the results manifested from DMD simulations suggested that the influence of single point substitution mutation on SOD1 protein that urged towards the increase in the formation of beta sheets (47%) were notably reduced upon the binding of hexapeptide (37%). Besides, the conformational preference of mutant protein was also being altered due to the binding of hexapeptide. Hence, we could suggest that the hexapeptide could act as an initial factor in designing the tripeptide for inhibiting the formation of aggregates by mutant SOD1.

3.2. De novo design of tripeptide from hexapeptide

With the results from the study above, we further embraced our study in computationally designing the tripeptides, which could act as a therapeutic regimen in inhibiting the formation of the aggregates in mutant SOD1 proteins.

3.3. Discerning key amino acids from hexapeptide - Study 2

Derived hexapeptide was subjected to alanine scanning in discovering the key amino acids by substituting alanine on every amino acid of hexapeptide and further, to utilize alanine scanned model

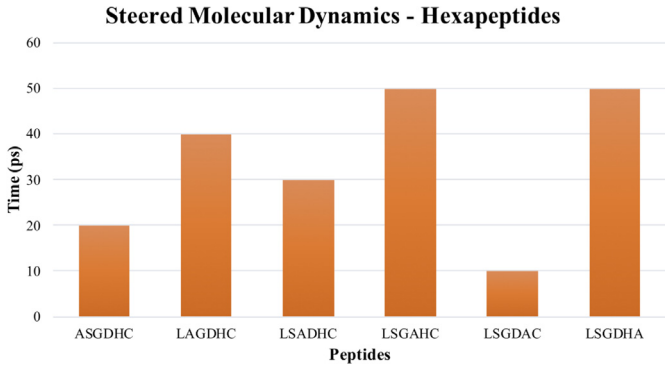

Fig. 1. SMD performed on the alanine scanned model peptides complex with mutant SOD1 protein portraying the time required to disintegrate from mutant protein with a distance of 25 Å.

peptides for molecular docking, SMD and DMD simulations approach. Initially, we performed the protein-peptide docking using HEXPEP program. The results from the program predicted that the binding affinity of alanine substituted hexapeptide at 1st, 3rd and 5th position was reduced (−99.94, −103.87 and −81.65 kcal/mol) relative to the other alanine substituted peptides at 2nd, 4th and 6th position with the binding affinity of −110.54, −115.64 and 108.39 kcal/mol, respectively. To further substantiate the aforementioned report on docking study, we incorporated SMD to pull off the alanine-substituted peptides to a distance of 25 Å from the mutant SOD1, using YASARA program (Fig. 1). The results from the Fig. 1 were corroborating with the results of docking studies, thus suggesting that the time period required to steer the alanine-substituted peptide at 1st, 3rd and 5th position on hexapeptide were lesser (20 ps, 30 ps, and 10 ps) as compared to the other alanine scanned peptides (40 ps, 50 ps and 50 ps). Therefore, we could establish that the substitution of alanine in place of Leu, Gly and His of hexapeptide, drastically altered the efficiency of binding with mutant SOD1. Although the results from docking and SMD studies were found to be satisfactory, the clarification to the inhibitory activity of alanine-substituted peptides on mutant SOD1 provide a clear representation and also, eloquently authenticate our findings. Thus, we performed the DMD simulation and the outcomes insinuated that the formation of beta-sheets in mutant SOD1 protein (47%) was found to be slightly reduced upon binding with alanine-substituted peptide in 1st, 3rd and 5th position of hexapeptide with 41%, 43%, and 44%, respectively. While the vice-versa was found at the other alanine substituted peptides with 35%, 38% and 34% on the mutant SOD1 protein (Fig. 2). Therefore, the results from DMD simulations suggested that the substitution of alanine over the amino acids Leu, Gly and His of hexapeptide utterly distorted the inhibitory effect of peptide over the mutant SOD1. Overall, with the results from docking, SMD and DMD,

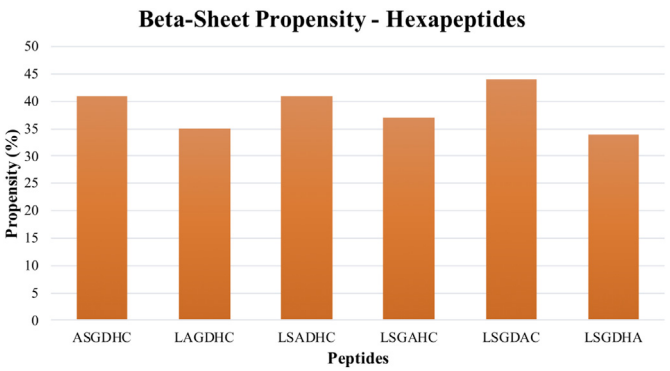

Fig. 2. Beta-sheet propensity of the mutant protein complexed with the alanine scanned peptides represented in the form of the bar graph.

we could propose that the amino acids L, G and H are the key factors in enhancing the inhibitory activity of the hexapeptide against the mutant SOD1 protein. Therefore, with the above finding suggesting that the critical amino acids (L, G and H) are required for the inhibition of the aggregate formation in the mutant SOD1, we rationally derived the tripeptide from the hexapeptide by designing the LGH residues. The tripeptide was designed as an initiative in providing the most efficient, potent and easily procurable peptide, which could have a greater inhibitory activity as compared to that of the hexapeptide.

3.4. Assessment on interactions between rationally designed tripeptide LGH and mutant SOD1- Study 3

Rationally designed tripeptide (LGH) was geometrically optimized, using MOPAC. The optimized tripeptide was utilized for further analyses. Primarily, we docked tripeptide with mutant SOD1, using HEXPEP. It was revealed that the binding region of tripeptide was similar to hexapeptide complexed with mutant SOD1. Moreover, the binding residues of tripeptide were also found to be similar to that of hexapeptide. Therefore, we retrieved the binding site residues and the interactions of tripeptide with mutant SOD1 (Table 1). Accordingly, the results determined that Lys3 and Glu21 exposed hydrogen bond interaction with Gly and Leu of tripeptide, respectively. Further, the hydrophobic interactions were seen with the residues Val4, Asn19, Phe20, Lys30, Trp32 and Gln153. Thereby, indicating that the tripeptide has significant interaction with the vital amino acids, which could be a prime cause for the stronger binding affinity with mutant SOD1. Most importantly, the binding site residues Val4, Asn19, Phe20, Lys30, Trp32 were established to be communal in both, the hexapeptide and tripeptide docked complex. Hence, we could suggest that the tripeptide shares a similar binding pocket and key binding residues as of hexapeptide with mutant SOD1. Besides, the results from the docking study contributed to an outlier conception on the interactive binding action of tripeptide with mutant SOD1. Thus, to further elucidate and corroborate the interactive affinity of tripeptide with mutant SOD1, we utilized SMD approach, in which the tripeptide was pulled off to a distance of 25 Å from mutant SOD1. The time period was computed to be 60 ps in order to steer the tripeptide to a non-interactive distance from mutant SOD1. Fascinatingly, the time period required to pull out the hexapeptide to a distance of 25 Å from mutant SOD1 was similar to tripeptide. Hence, our results were more authenticate in protruding that the computationally designed tripeptide LGH could have a similar geometrical binding description as SOD1 derived hexapeptide. Furthermore, we attempted to postulate an endorsing report on the inhibitory effect of tripeptide on mutant SOD1 protein over the dynamic time scale, using DMD. The outcomes established from the DMD simulations inferred that the increase in the formation of beta-sheets in mutant SOD1 protein with 47% was substantially reduced (36%) upon interacting with tripeptide. The results from the tripeptide study were marginally similar to that of the hexapeptide study. With these provoking results, we could substantiate that our computationally designed tripeptide could be a successful candidate in designing new peptides with more efficiency on inhibiting the aggregate formation in the mutant SOD1 protein.

3.5. Empirical restructuring of LGH tripeptide on improving the disaggregation efficacy against mutant SOD1 - Study 4

We computationally redesigned the tripeptide (LGH) in order to improvise its binding affinity and the inhibitory activity against mutant SOD1. Here, we considered two most important key features, the torsion angle and energetic factor in redesigning the tripeptide, using CUPSAT program. It was inferred that Trp and Cys were exhibiting the more favorable and stabilizing energy upon substitution of Leu of tripeptide. With these results, the two new tripeptides, WGH and CGH were designed accordingly. Further, a similar trend was followed for

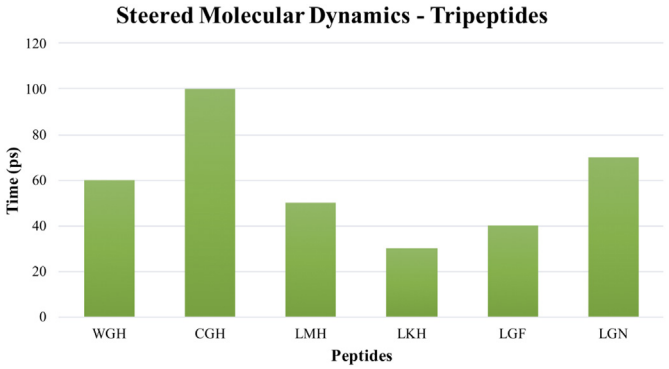

Fig. 3. Bar graph representation of the SMD performed on the de novo designed peptides complex with mutant SOD1 protein to disintegrate from mutant protein to a distance of 25 Å.

Gly and His tripeptide residues, which upon substitution with Met, Tyr, Phe and Asn showed favorable and stabilizing energy. Consequently, the outcomes incite towards the design of LMH, LKH, LGF and LGN from the initially designed tripeptide. From the above-monitored study, we rationally designed six new tripeptides (WGH, CGH, LMH, LKH, LGF and LGN) from our candidate tripeptide (LGH). The designed peptides were further optimized, using MOPAC program. The optimized six tripeptides were further docked with mutant SOD1, using HEXPEP. Therefore, we accessed the binding affinity of those six tripeptides complexed with mutant SOD1, using SMD approach, in which, each of the tripeptides was pulled off to a distance of 25 Å from mutant SOD1. The time scale essential to disassociate from mutant SOD1 was computed for all the tripeptides (Fig. 3). It was deciphered that the time required to pull off the tripeptide CGH was greater (100 ps) than all other tripeptides WGH (60 ps), LMH (50 ps), LKH (30 ps), LGF (40 ps) and LGN (70 ps), considerably. With the results from SMD, we could only stipulate an evocative conjecture on the binding affinity terms of the six tripeptides with mutant SOD1. However, we also determined the inhibitory activity of those six peptides against mutant SOD1, using DMD simulation for ascertaining a reminiscent conclusion. It was suggested that the propensity of beta-sheet (Fig. 4) formed in mutant SOD1 (47%) was significantly reduced upon binding with CGH tripeptide (32%) followed by LKH (35%) < LMH (36%) < LGN (36%) < WGH (38%) < LGF (40%). Therefore, we could suggest that CGH had a greater inhibitory effect rather than the other newly designed tripeptides, substantially. With the outcomes from both, the SMD and DMD studies, we could conjecture that CGH has stronger interactions (Table 1) and greater inhibitory activity against mutant SOD1 as compared to all the other tripeptides reported in our study.

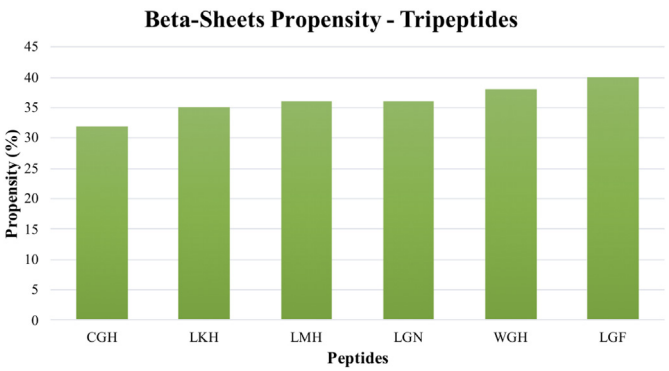

Fig. 4. Graphical picturization of the beta-sheet propensity in terms of bar graph for the mutant protein complexed with the de novo designed tripeptides.

3.6. Conclusive association from the reported studies

To postulate an evident result from our studies, we broadened our study by examining the effect of hexapeptide, LGH and CGH peptides on mutant SOD1 regarding the conformational preferences that influence towards the change in the geometrical architecture of mutant SOD1.

3.7. Binding free energies over conformational sampling

To endorse the precision of binding, we calculated the binding free energy of mutant complex with hexapeptide, LGH and CGH tripeptides. The preeminent docked complex was utilized for conformational sampling (Fig. 5). Trailed by, the conformers obtained from the conformational sampling approach were used to compute the binding free energies of all the complexes using PRODIGY (Fig. 6). It was elucidated that the mutant-hexapeptide complex possesses the average binding energy of −5.61 kcal/mol, while the average binding free energy of LGH tripeptide with mutant SOD1 was −4.91 kcal/mol. Interestingly, the computationally designed new tripeptide, CGH was found to have an overall binding free energy of −5.35 kcal/mol with mutant SOD1. Moreover, the results agreed with the SMD studies (Fig. 7), where the overall time period required to pull off the hexapeptide and the tripeptide LGH were marginally similar with 60 ps. Whereas, the time scale to unbind the CGH tripeptide was significantly greater (100 ps) than both, the hexapeptide and tripeptide. Thus, the fallouts from the binding free energy and SMD approaches suggested that the newly designed tripeptide, CGH could be more reliable in terms of the binding with the aggregated mutant SOD1. To rationalize the overall effect of these peptides on the aggregated mutant SOD1, we further characterized distinct geometrical analysis to support our finding in a noteworthy factor.

3.8. Geometrical analysis over conformational time scale

To provide a comprehensive analysis, we computed the residual flexibility for c-alpha carbon atoms of mutant and mutant-complex using g_rmsf utility present within the GROMACS program (Fig. 8). The results from the residual flexibility exemplify the flexibility of specific residues around its mean position, thus specifying the categorized report over the dynamical stability of SOD1. Further, we examined the conformational stability using the residual flexibility values imperceptibly. Fig. 8 characterized the impact of the three peptides on the residual flexibility comparative to mutant. By our earlier reports, we could conjecture that A4V mutation has profoundly biased the total residual flexibility of SOD1. Contrariwise, the results were found to be vice-versa upon binding of hexapeptide and tripeptides with mutant SOD1. Precisely, the aggregation eliciting fragments in mutant SOD1 (141–145) that presented lesser residual flexibility was discovered to have greater flexibility, when bounded with tripeptides as compared to hexapeptide. Besides, the calculated average residual flexibility of mutant and its complex with hexapeptide, LGH and CGH were found to be 0.17 nm, 0.18 nm, 0.20 nm and 0.20 nm, respectively. Accordingly, the results from the flexibility of the residues could associate with the conformational stability of SOD1, by denoting that the augmented flexibility in mutant complex could be primely due to the loss in secondary structural propensity as of mutant SOD1. Further, we analyzed the RMSD and Rg parameters that govern the overall conformational deviation and compactness of the protein. The obtained results portrayed that the impact of mutation has altered the conformational deviation (Supplementary Fig. 1) and compactness (Supplementary Fig. 2) of SOD1 protein, which upon binding with CGH was found to be reduced as compared to that of hexapeptide and LGH. Besides, the results from the geometrical studies were also found to be statistically significant. In order to further substantiate the outcomes from the geometrical studies, we computed the overall and per residual secondary

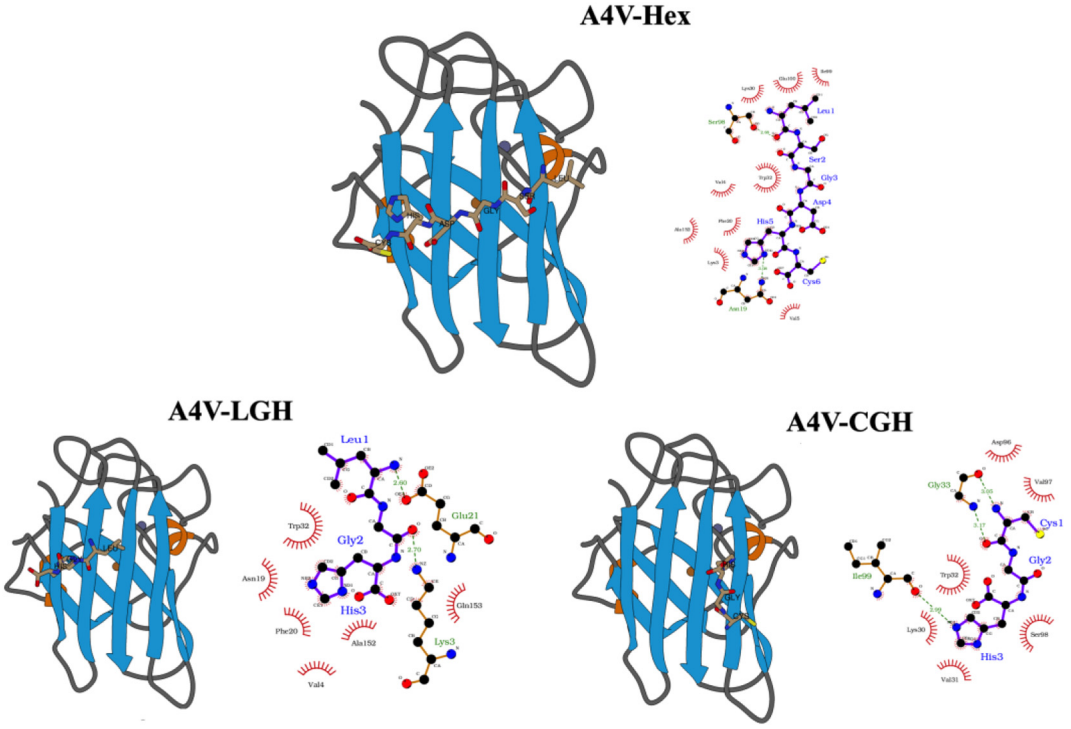

Fig. 5. Molecular visualization of the docked complex and key interactions of the mutant SOD1 protein with the hexapeptide (Hex), LGH and CGH tripeptides.

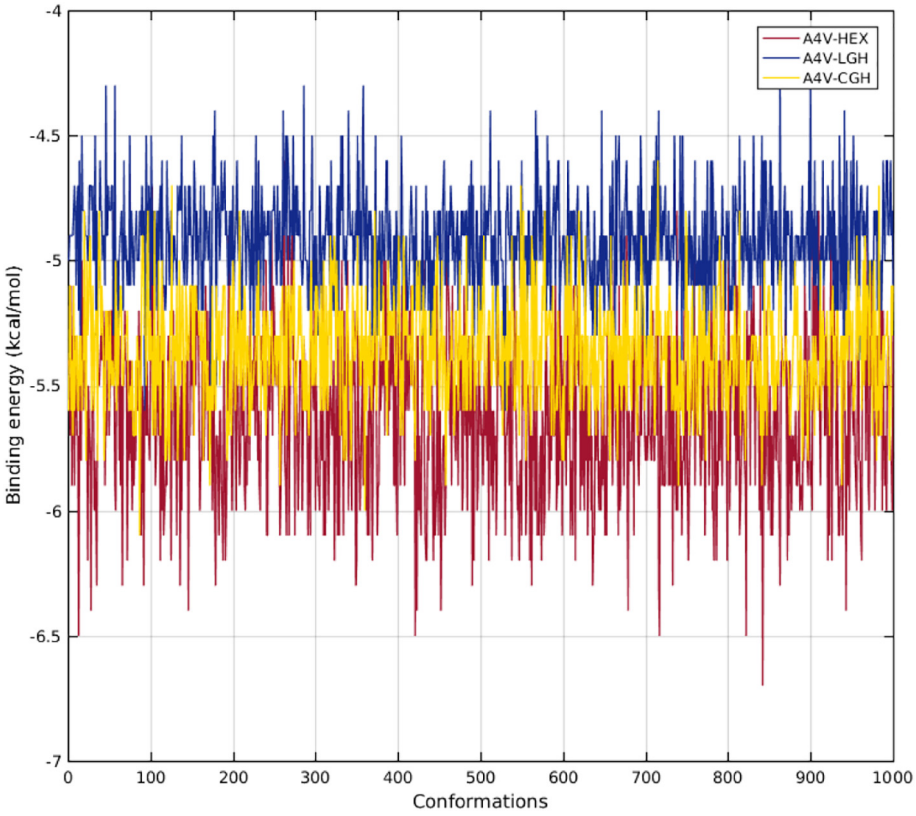

Fig. 6. Binding free energy of the mutant complexed with hexapeptide (Hex), LGH and CGH peptides over the sampled conformations.

structure propensity for A4V, A4V-Hex, A4V-LGH and A4V-CGH proteins, correspondingly.

3.9. Secondary structure propensity of mutant and mutant peptide complex

Evidence from varying studies has stipulated that the tendency of the helical and beta-sheet structures are the key factors that unveil the physiological function and the aggregation property of the protein [65].

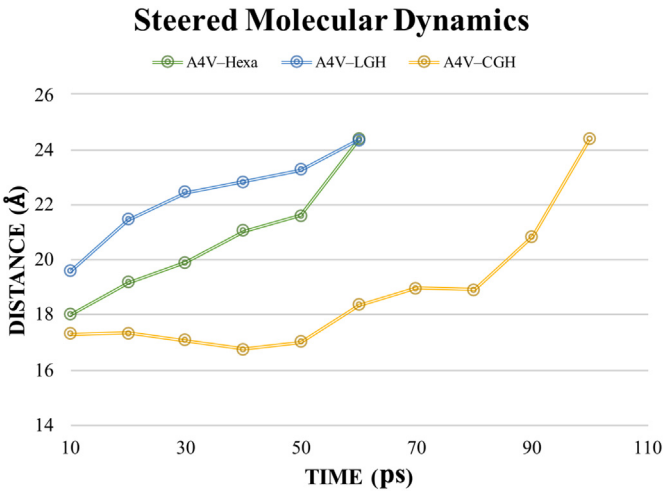

Fig. 7. Graphical representation of the time period over the SMD to pull off the hexapeptide (Hexa), LGH and CGH peptides to a distance of 25 Å from the mutant SOD1 protein.

In consideration with that, we computed the complete secondary structure content for mutant and the three other mutant-peptide complex, via DSSP program (Fig. 9). It was found that the mutation in SOD1 from Ala to Val at 4th position led to the loss of alpha helix propensity, which was found to be regained upon binding with peptides in the subsequent grading of hexapeptide (1%) < LGH (4%) < CGH (4%). Consequently, we envisaged noticeable variations in secondary structure propensities of beta-sheets upon binding of hexapeptide, LGH and CGH peptides, correspondingly. It was inferred that the propensity of beta-sheets in mutant (48%) reduced significantly upon binding with CGH (32%) followed by LGH (35%) and finally, the hexapeptide with 36%. Contradicting to the aforementioned trend, the propensity of coil and bend in mutant complex with hexapeptide (28% and 21%), LGH (32% and 16%) and CGH (34% and 17%) were augmented as compared to mutant SOD1 (22% and 15%). Besides, the structure of turn in mutant and its complex forms with hexapeptide and tripeptides (LGH and CGH) exhibited a marginal difference with 1%. Hence, we determined that the reduced percentage of beta-sheets in mutant complex could be transformed into helix, bends and coils, correspondingly (Supplementary Table 1).

To specify a detail report on secondary structure transformation, we calculated the secondary structural changes over the residues of mutant and its complex with hexapeptide and tripeptides over the entire period of simulation (Supplementary Fig. 3). Curiously, the outcomes shed lights on the influence of peptides on mutant SOD1. In comparison with our earlier reports on wild type and mutant SOD1 (A4V), we could conjecture that the plentiful beta-sheets structures are formed in the regions of 41–44, 60–63, and 83–88 with a probability greater than 0.75. Relatively, the abundance probability of beta-sheets formed in mutant was reduced upon binding with hexapeptide at residual positions of 41–43, 48–53, 55–63, 83–89 and 120–123. A similar trend of the results was seen in mutant on interacting with LGH tripeptide in addition to that the residues positioned at 142–144 exhibited a lesser probability of beta-sheet formation. Further, the binding of CGH peptide on mutant SOD1 has significantly reduced the probability of beta-sheet formation in the residues located at 42–44, 49–52, 53–56, 83–88, 120–122 and 142–144. Thus, it was stipulated that the binding of tripeptides aided in reducing the beta-sheet formation mostly in aggregation-prone regions that are reported from the experimental studies [66]. It was determined that the residues forming beta-sheets with a probability of 0.75 and above in mutant protein was reduced upon binding with CGH > LGH > hexapeptide. Furthermore, the probability of helical structure is marginal in mutant SOD1 which exhibited augmented probability upon binding with CGH, LGH and hexapeptide in the residual region positioned at 124–128. Therefore, the complexation of LGH and CGH had deteriorated the tendency of mutant to

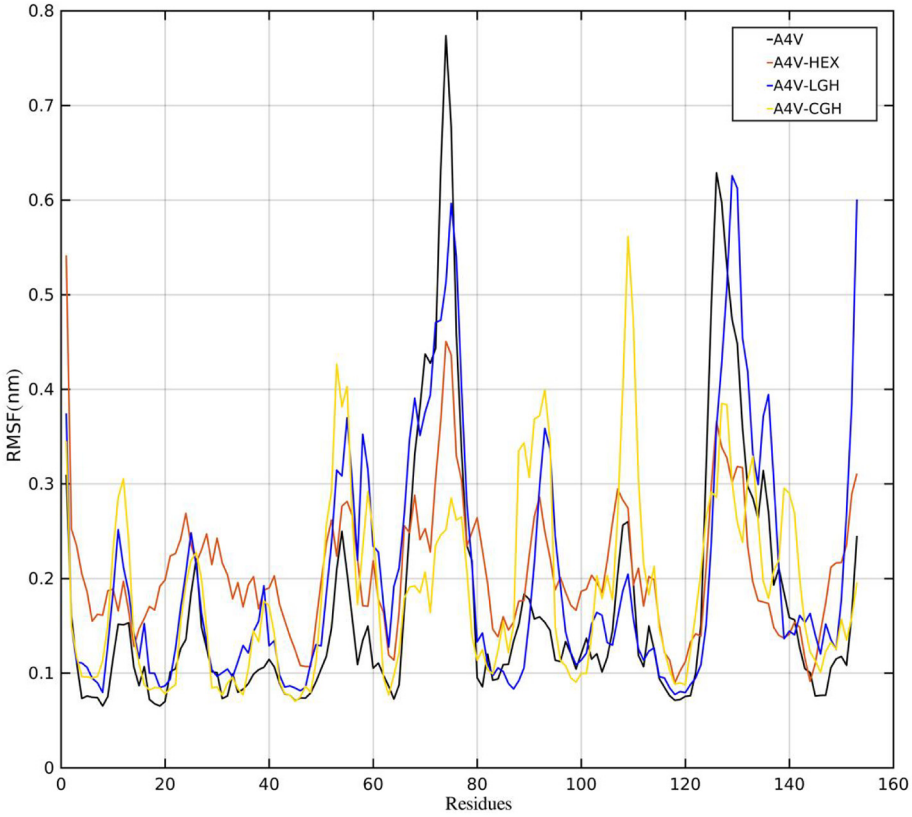

Fig. 8. Conformational flexibility of the mutant SOD1 protein in its apo and complex states.

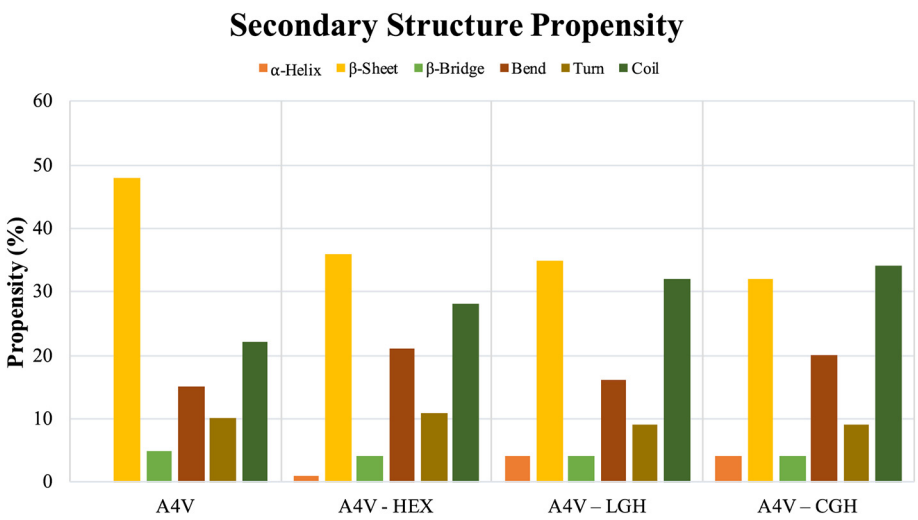

Fig. 9. Secondary structural propensity of the mutant protein in apo and complexed state computed over the time scale of molecular simulation.

acquire beta-structure and developed its inclination to form the helix. Besides, the configuration of bends in mutant, with a probability above 0.75 was found to be augmented upon binding with CGH followed by LGH and hexapeptide. Furthermore, similar trends of the results from the structural bends were seen in the coil structure of mutant SOD1 thereby suggesting that the richness of coil in mutant was established to increase upon interacting with CGH and LGH relative to hexapeptide. Thus, the residual secondary structural probability pictured that the mutant complex with increased probability of the helix, bend and turn content urged towards the loss in beta-sheet probability that could be the root cause for the altered conformation and increased flexibility. With the outcomes from the secondary structural variations, we could suggest that the binding of CGH > LGH > hexapeptide could condense the formation of aggregates in mutant SOD1.

3.10. Assessing the formation of the aggregates using free energy landscape

To provide an obvious result regarding the disaggregation effect of computationally designed tripeptides in comparison with hexapeptide on mutant SOD1, we mapped the 3-D free energy landscape (FEL) of conformational ensembles concerning RMSD and Rg parameters for the mutant and mutant-peptide complex, using g_sham (Fig. 10). The free-energy funnel of mutant and its complex states exhibited relatively distinct shapes. The FEL of mutant conformations exhibits multiple large basins. The broad basins were positioned within the Rg and RMSD values of 1.58 nm and 0.35 nm respectively. The wide basins with multiple free energy points indicated the formation of aggregated mutant SOD1 conformations. On the other hand, the binding of hexapeptide influenced this trend with the free energy basin located at Rg and RMSD values of 1.60 nm and 0.45 nm correspondingly. Interestingly, the FEL basin plotted for mutant LGH complex conformers were seen to be confined with the minimal region of Rg and RMSD with 1.52 and 0.30 nm, individually. More interestingly, the interaction of CGH with mutant SOD1 throughout the conformational dynamics showed that the free energy basin was confined to one particular region and those regions were discovered to have Rg and RMSD values of 1.44 nm and 0.25 nm, respectively. Thus, the outcomes from FEL studies signified that the binding of CGH aid in reducing the unfavorable free energy basins formed by the mutant SOD1 protein. Thus, it was concluded that the misfolded structures obtained by mutant were reduced upon binding with CGH peptides. Furthermore, the FEL from other studies has established that upon aggregation protein acquire numerous FEL basins, which support our results subtly [20–23]. Hence, the irrecoverable variations in mutant structures elevating the toxic aggregates formation were hampered upon binding with CGH, thereby considerably altering the FEL.

4. Conclusion

Future of drug discovery is being focused on the use of peptides for treating various neurological disorders since most of the small molecules fail to pass the blood-brain barrier. Therefore, we intend to suggest the valuable use of SOD1 derived peptide in designing the tripeptide (CGH) that could effectively hinder the formation of the aggregates in mutant SOD1. To design the tripeptide, we utilized a new cutting-edge methodology with the aid of computerized algorithms and programs in the follow up of molecular docking, binding affinity, tCONCOORD, SMD, DMD and free energy landscape studies. Initially, with the use of alanine scanning, we designed the LGH tripeptide, which was found to have an inhibitory effect relative to hexapeptide. However, we rationally used the torsional angle studies and energetic parameters in consideration for designing the new tripeptide CGH with the balanced structural state. Overall, the results from our study stipulated a substantial influence of CGH peptide against mutant SOD1 that significantly inhibited the formation of aggregates relative that of LGH and hexapeptide. To substantiate further, we analysed the proteolytic stability of CGH, LGH and Hexapeptide from peptide cutter program. With the outcomes, we could infer that chymotrypsin with low specificity alone showed a cleavable site in CGH peptide whereas, pepsin, proteinase, formic acid and ASP-N endopeptidase showed cleavable sites in LGH and Hexapeptide. Hence, the proteolytic cleavage of the CGH was found to be lesser than that of LGH and hexapeptide. Hence, our study could be the first of its kind in designing tripeptides against the mutated SOD1, thus paving the way for the futuristic design of peptides against the amyloid-related diseases affecting the human all over the globe.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgement

The authors thank Vellore Institute of Technology, Vellore for providing 'VIT SEED GRANT' for carrying out this research work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2019.116425.

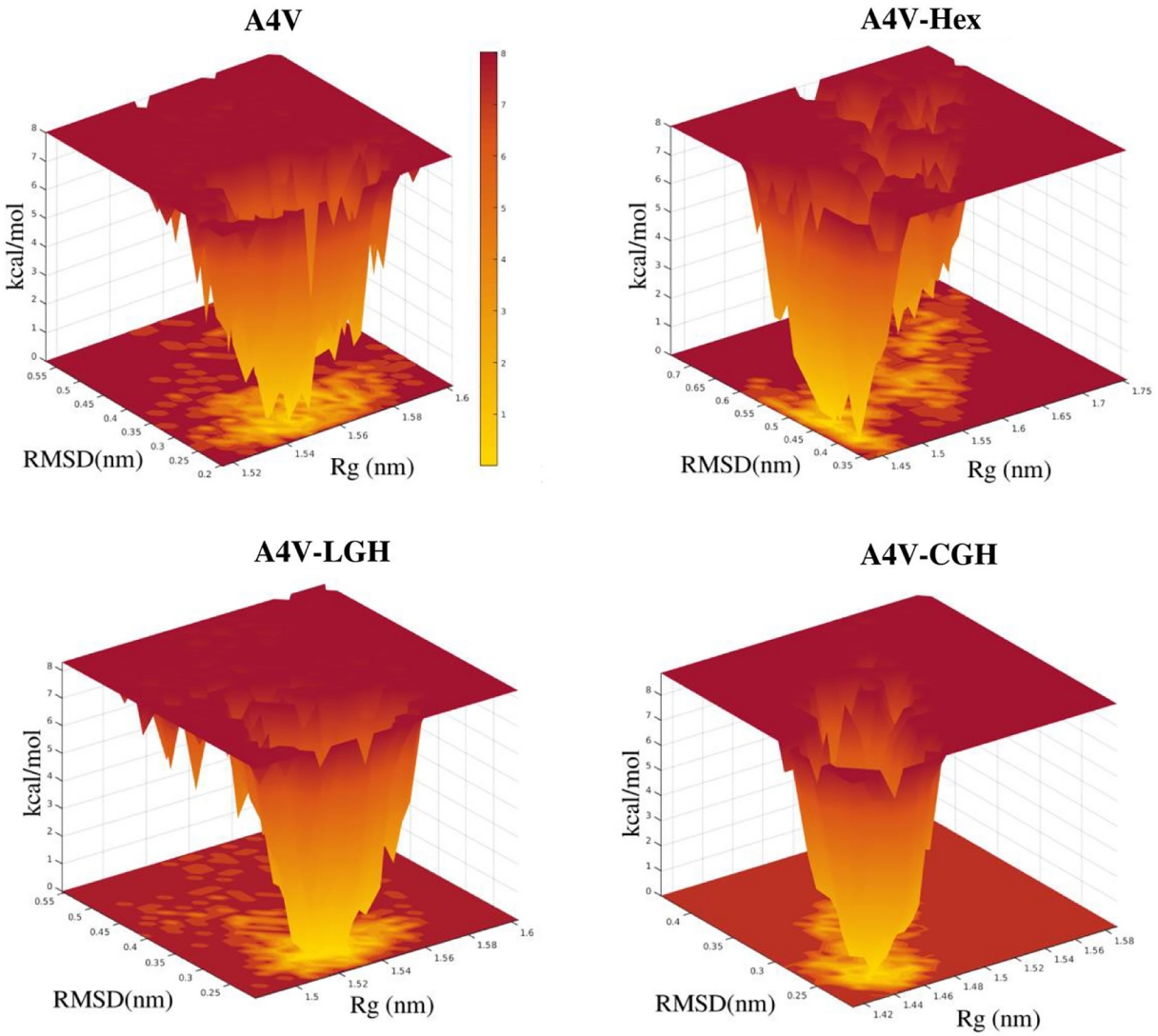

Fig. 10. Free energy landscape of the mutant SOD1 protein and its complex state with hexapeptide (Hex), LGH and CGH peptides over the period of simulation time.

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