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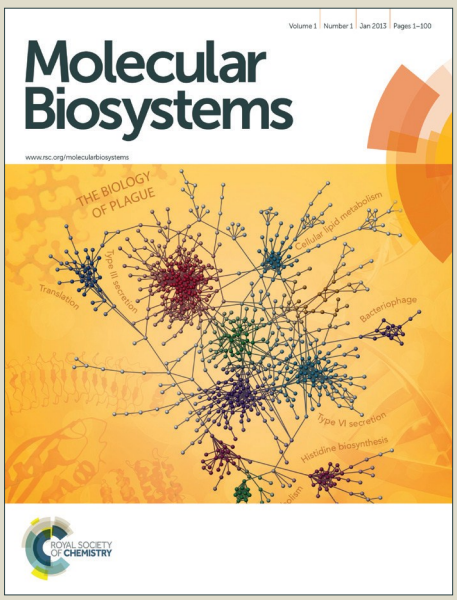

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Molecular modeling approach to explore role of Cathepsin B from *Hordeum vulgare* in degradation of Aβ peptide

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Abstract

The pathological hallmark of Alzheimer disease is accumulation of Aβ peptides in human brains. These Aβ peptides can be degraded by several enzymes such as hACE, hECE, hIDE and cathepsin B. Out of which cathepsin B also belongs to papain super family and has been found in human brains having role in Aβ peptide degradation through limited proteolysis. The Aβ concentrations are maintained properly by its production and clearance via receptor-mediated cellular uptake and direct enzymatic degradation. However, reduced production of Aβ degrading enzymes as well as their Aβ degrading activity in human brain initiates the process of accumulation of Aβ peptides. So it becomes essential to investigate molecular interactions involved in the process of Aβ degradation in detail at the atomic level. Hence, homology modeling, molecular docking and molecular dynamics simulation techniques have been used to explore the possible role of cathepsin B from *Hordeum vulgare* in the degradation of amyloid beta (Aβ) peptide. The homology model of cathepsin B from *Hordeum vulgare* shows good similarity with human cathepsin B. Molecular docking and MD simulation results revealed that active site residues Cys32, HIS112, HIS113 are involved in catalytic activity of cathepsin B. The sulfhydryl group of Cys32 residue of cathepsin B from *Hordeum vulgare* cleaves Aβ peptide from carboxylic end of Glu11. Hence, this structural study might be helpful to design alternative strategies for the treatment of AD.

Keyword: Cathepsin B from *Hordeum vulgare,* Alzheimer disease, Homology modeling, Molecular docking, Molecular dynamics.

Abbreviations

AD- Alzheimer's disease

Aβ peptide- Amyloid Beta Peptide

Plant Cathepsin B- Cathepsin B from *Hordeum vulgare*

MD- Molecular Dynamics

RMSD- Root Means Square Deviation

Introduction

Cathepsin B belongs to the peptidase family having important role in intracellular protein degradation. The proteins entering in the endolysosomal system is degraded by cathepsin B[1]. Amyloid beta (Aβ) peptides play a central role in pathogenesis of Alzheimer's disease its accumulation in AD brains is due to overproduction or insufficient clearance and the defects in the proteolytic degradation[2-3] Aβ (1-42) peptide appears to be particularly critical in AD pathogenesis[4-6]. The familial autosomal dominant (FAD) mutations and the defects in the proteolytic degradation results into increase the production or relative abundance of Aβ (1-42) in AD brains[7-8]. Hence, degradation and clearance of Aβ peptide could be promising therapeutic approach for the treatment of AD. Cathepsin B reduces Aβ levels by inducing C-terminal truncation of Aβ (1–42) which suggest that endogenous cathepsin B activity could reduce levels of Aβ, especially Aβ1–42, and protect against AD-related deficits[9]. ACE, ECE, IDE, neprilysin, plasmin, and MMP-9 are the enzymes which have been implicated in cerebral Aβ clearance[10-14]. Reducing inhibitor levels such as cysatin C can also enhance cathepsin B enzymatic activity[15]. The persons suffering from neurodegenerative pathologies show abnormal distribution of cathepsin B and its endogenous inhibitors[16]. Studies showed that balance between cathepsin B and its endogenous inhibitors (secretory cystatin C and cystatin B) are important for dissolution of amyloid plaques and toxic oligomers clearance[17]. The human cathepsin B has certain limitations such as mutation, effect of inhibitors and direct oxidative damage due to which low cathepsin B activity was seen[18]. So there is a need to find the enzyme from novel source which can be used to degrade Aβ peptide and control its deposition.

Hence, the structure of cathepsin B from plant source *Hordeum vulgare* showing high homology with human cathepsin B[19] could be useful to find out degradation mechanism of Aβ peptides by plant cathepsin B which can be useful to develop new strategies against AD. So that we have developed three-dimensional structure of cathepsin B from a plant source *Hordeum vulgare* using Modeller 9V7 software. The energy minimization of predicted model was done by steepest descent method to remove steric clashes. Structure validation is also done through various online servers which confirm good quality of predicted structure. The sequence analysis and 3D model comparison between plant cathepsin B from *Hordeum vulgare* and human cathepsin B shows high homology with each other. The molecular dynamic simulation confirms stability of predicted model. Molecular docking and MD simulation studies revealed similar

structural fold between plant cathepsin B and human cathepsin B. Hence, predicted model of cathepsin B from plant source could be important in studies of Aβ peptide degradation.

Methods

Software used for the prediction and validation of three dimensional structures

The three dimensional model of plant cathepsin B from *Hordeum vulgare* was built by using SWISSMODEL[20], Geno3D[21], and MODELLER 9v7[22]. Then evaluation of models was done by PROCHECK[23], PROSA[24] and Verify-3D[25]. Molecular docking study was performed with AutoDock 4.2[26]. Structure visualization and analysis were carried out using chimera[27].

Sequence alignment, Secondary structure analysis and homology modeling

Homology model of Cathepsin B from *Hordeum vulgare* was built by using Human cathepsin B as a template. The FASTA sequence of Cathepsin B from *Hordeum vulgare* was retrieved from NCBI protein sequence database (Accession No- BAK03491.1). The BLAST program was used to search suitable template available in the PDB. Finally the Cathepsin B from human (PDB ID: 2IPP) with a resolution of 2.15Å was used as a template to build the model. The pairwise sequence alignment of the template sequence (2IPP) and the Cathepsin B from *Hordeum vulgare* sequence was done using the EMBOSS program[28]. Then Secondary structure analysis of Cathepsin B sequence from *Hordeum vulgare* was done by "SOPMA" (Self-Optimized Prediction Method) program[29]. Finally homology modeling of Cathepsin B from *Hordeum vulgare* was performed by three homology modeling programs; SWISS-MODEL[20], Geno3D[21] and Modeller9V7[22]. The steepest descent energy minimization method[30] was used to remove steric clashes.

Three dimensional model refinement and Validation

The models obtained by SWISS-MODEL[20], Geno3D[21] and MODELLER 9_V7[22] was validated by inspecting the Ramachandran plot[31] obtained from PROCHECK analysis[23]. After analysis of model derived from SWISS-MODEL[20], Geno3D[21] and MODELLER 9_V7[22] the model constructed by MODELLER was finally chosen for further investigations on the basis geometry and 3D alignment with template.

The PROSA[24] server analysis is carried out to check the energy criteria in comparison with the potential of mean force derived from a large set of known protein structures. The second test was done to apply energy criteria using ProSA II energy plot to investigate whether the interaction energy of each residue with the remainder of the protein is negative. Further, the compatibility of the model with its sequence was measured by Verify-3D[25]. In Verify-3D[25] the vertical axis represents the average 3D–1D protein score for each residue in a 21 residue. Further, the Ramachandran plot was obtained through PROCHECK analysis[23]. The predicted model is compared with template structure by using PDBeFOLD server[32] and also by using chimera[27].

Prediction of active site

The active site prediction in the refined model was done by automatic sequence alignment. We aligned the sequences of the Cathepsin B from *Hordeum vulgare* with the sequence of Human Cathepsin B (2IPP) for which the active site is known[19]. The 3D alignment between Cathepsin B from *Hordeum vulgare* and Human Cathepsin B (PDBID-2IPP) was done by chimera[27] to compare the active site pocket.

Molecular dynamic simulation of predicted model of cathepsin B form *Hordeum vulgare*

Molecular dynamic simulation was performed using Gromacs 4.0.4 program[30] by OPLS-AA force field[33-34]. The homology model of Cathepsin B from *Hordeum vulgare* built by modeler was used as a starting model for MD. The complex was surrounded by 0.9 Å layer of water and system was neutralized with 14 Na+ ions. The solvated structure was minimized by steepest descent method for 5730 steps at 300K temperature and constant pressure followed by equilibration. After equilibration production MD was run for 5ns at 300k. The PME algorithms were used during simulation[30].

Molecular docking of simulated model of plant Cathepsin B and Aβ peptide

The molecular docking has been performed between simulated model of plant Cathepsin B and patch of Aβ peptide (^{10}YDVHHNKLVFF^{20}) from 1AML.pdb[35] using AutoDock 4.0[26]. Initial receptor and ligand structures were minimized by steepest descent (SD) method using Gromacs 4.0.4 to remove internal strain energy[30]. Lamarckian genetic algorithm (LGA) has been used in this study. The active site was defined using Auto Grid[26]. The grid size was set to 48 × 44 × 54 points with a grid spacing of 0.375 Å centered on the selected flexible residues present in

the active site of plant Cathepsin B. The grid box contains the entire binding site of the Plant Cathepsin B and provides enough space for Aβ peptide for the translation and rotation. Step size of 2 Å for translation and 50º for rotation were chosen and the maximum number of energy evaluation was set to 25, 00,000. Thus, ten runs were performed. For each of the 10 independent runs, a maximum number of 27,000 GA operations were generated on a single population of 150 individuals.

Similarly, whole Aβ peptide (1AML.pdb) also docked with plant cathepsin B using AutoDock 4.0[26]. The same protocol was followed as per earlier docking between plant Cathepsin B and patch of Aβ peptide. The same rigid and flexible files were used while the whole Aβ peptide was taken as a ligand. The grid size was set to 50× 50 × 50 points with a grid spacing of 0.375 Å centered on the selected flexible residues present in the active site of plant Cathepsin B. The remaining parameters were kept similar to earlier docking.

MD simulation of docked complex of simulated model of Cathepsin B from *Hordeum vulgare* and Amyloid beta peptide

Further the docked complex of cathepsin B *Hordeum vulgare* and Aβ peptide ^{10}YDVHHNKLVFF^{20} was simulated with Gromacs 4.0.4 program[30] by OPLS-AA force field[33-34]. The complex was surrounded with 0.9 Å layer of water and system was neutralized with 14 Na+ ions. The solvated structure was then minimized by steepest descent method for 3704 steps at 300K temperature and constant pressure followed by equilibration. After equilibration production MD was run for 50ns at 300k. The PME algorithms were used during simulation[30]. All the MD simulation studies were performed on HP Workstation. Structural comparison between initial structure and final structure of MD simulation was done using PDBeFOLD[32]. All the dynamic runs were then visualized using the VMD (Visual Molecular Dynamics) package[36]. Images after simulation were generated using chimera[27] and PyMOL. Then structure of Cathepsin B from *Hordeum vulgare* and its docked complex with Aβ peptide ^{10}YDVHHNKLVFF^{20} was analyzed by various software like "RasMol"[37] "PyMOL" (http://PyMOL. sourceforge.net/) and "Chimera"[27].

Results and discussion

The secondary structure analysis of cathepsin B sequence from *Hordeum vulgare* by "SOPMA" method shows 18.22%, 54.65%, 17.44%, 9.69% amino acids residues in the form of a helix, random coil, extended strand and beta turn respectively (Supplementary Fig. 1). The SOPMA analysis gives secondary structure elements which showed that random coil occupied the largest part of the protein followed by alpha helix, extended strand and beta turns for plant cathepsin B (Supplementary Fig. 1). The structure analysis was done by PDBsum server[38] which also showed that random coil occupied the largest part of the protein followed by alpha helix, extended strand and beta turns (Fig. 1 and Supplementary 2). There was 56% alignment **(**Fig. 2**)** between the target sequence of cathepsin B from *Hordeum vulgare* and the sequence of human cathepsin B (2IPP.pdb)[19]. The PROCHECK[23] validation was done for all the models of cathepsin B from *Hordeum vulgare* obtained by Geno3d (Table 1). Further the three dimensional model of Cathepsin B from *Hordeum vulgare* was predicted through Swiss model server. The PROCHECK[23] analysis was done for model predicted by Swiss model server (Table 1). Finally the three-dimensional structure of Cathepsin B from *Hordeum vulgare* was modeled by comparative protein modeling methods by using MODELLER 9_V7 software[22]. The stereo chemical quality of the Cathepsin B models was evaluated with the help of Ramachandran plot by PROCHECK[23]. Based on this plot the model contained 87.9% residues in most favored regions, other 10.7% of amino acids were present in allowed regions and only 1.4% of residues in disallowed regions, the total 98.6% of residues are present in favored regions (Supplementary Fig. 3)**.** Hence, PROCHECK[23] analysis results are better for model predicted by MODELLER 9_V7[22] rather than SWISS MODEL[20] and Geno3D[21]. Another test ProSA[24] was done for additional confirmation of good quality of cathepsin B model from *Hordeum vulgare* predicted by MODELLER 9_V7[22]. The Z score given by ProSA[24] for cathepsin B from *Hordeum vulgare* predicted by MODELLER 9_V7[22] was -6.59, which is in the range of native conformation and also the point given by ProSA[24] tool was found within the acceptable range of X-ray and NMR studies (Fig. 3A). The analysis of cathepsin B model from *Hordeum vulgare* by PROSA[24] showed negative interaction energy for maximum residues whereas very few residues displayed positive interaction energy (Fig. 3B). The structure of cathepsin B from *Hordeum vulgare* built by MODELLER[22] when compared with template structure using PDBeFOLD[32] and chimera[27] showed good similarity (Fig. 4B)**.** The sequence alignment and 3D comparison of cathepsin B

from *Hordeum vulgare* with human cathepsin B (2IPP)[19] shows that the active site residues (CYS32, HIS112, and HIS113) of cathepsin B from *Hordeum vulgare* exactly similar to active site residues (CYS29, HIS110, and HIS111) of human cathepsin B (Fig. 2 and 4B)**.** The active site pocket of cathepsin B from *Hordeum vulgare* also found similar to human cathepsin B (2IPP)[19] (Fig. 4B)**.** Therefore the residues CYS32, HIS112, and HIS113 might act as active site residues for cathepsin B from *Hordeum vulgare.*

Molecular Docking and MD Simulation studies of cathepsin B and Aβ peptide complex:

Molecular docking and MD simulation studies have been found useful to understand protein and ligand interactions in detail at the molecular level[39-46]. The residues such as (CYS32, HIS112, and HIS113) of cathepsin B from *Hordeum vulgare* were kept flexible and then docked with patch of Aβ peptide (1AML.pdb) [10]YDVHHNKLVFF[20]. The docked complex was analyzed before MD which shows that the residue CYS32 of cathepsin B from *Hordeum vulgare* interacts with GLN11 of patch of Aβ peptide ($A\beta_{10-20}$) by interatomic distance of 2.503 Å (Fig. 5A, Table 2). The docked complex of Cathepsin B from *Hordeum vulgare* with whole Aβ peptide ($A\beta_{1-40}$) showed similar type of interactions as found in the docked complex of cathepsin B with patch of Aβ peptide ($A\beta_{10-20}$) **(**Fig. 5A, 6 and 7). The CYS32 of cathepsin B from *Hordeum vulgare* interacts with GLN11 of whole Aβ peptide ($A\beta_{1-40}$) by interatomic distance of 2.697 Å (Fig. 7, Table 3).

Further, the docked complex of cathepsin B from *Hordeum vulgare* with patch of Aβ peptide was simulated for 50ns. The average RMSD is 0.28 nm which shows stable behaviour of docked complex of cathepsin B from *Hordeum vulgare* and patch of Aβ peptide over the 50ns simulation period (Fig. 8). The results for Rg (Radius of gyration) also indicate stable behavior throughout the whole simulation time (Fig. 9). Dynamics of the residues can be identified by RMSFs calculated for the C-alpha atoms (Fig. 10). The RMSF results show small fluctuations for the rigid structural elements and larger fluctuations for the ends and loops (Fig. 10). Generally these results indicate that the simulation was stable over the entire simulation time. Thus, the interaction between CYS32 of cathepsin B from *Hordeum vulgare* with the GLN11 of Aβ peptide was maintained throughout MD simulation trajectory (Fig. 5B, 6, 7 and Table 2) which suggests that cathepsin B from *Hordeum vulgare* might cleave Aβ peptide at Glu11 similar to human cathepsin B[47].

Conclusions

The three dimensional structure of cathepsin B from *Hordeum vulgare* was predicted by using homology modeling technique. The sequence and structure analysis revealed that active site residues of cathepsin B from *Hordeum vulgare* are exactly similar to human cathepsin B. Molecular docking results showed proper interactions between sulfhydryl group of Cys 32 of cathepsin B with backbone carboxyl oxygen atom of Glu11 of Aβ peptide. This hydrogen bonding interaction between Cys32 of plant cathepsin B with carboxylic end of Glu11 has been maintained throughout MD simulation which suggests its probable involvement to cleave Aβ peptide. Hence, the structure of cathepsin B from *Hordeum vulgare* could be useful to study Aβ peptide degradation in detail at the atomic level. Thus, this study might be useful to understand amyloid beta peptide degradation as well as to design alternative strategies to control Alzheimer's disease.

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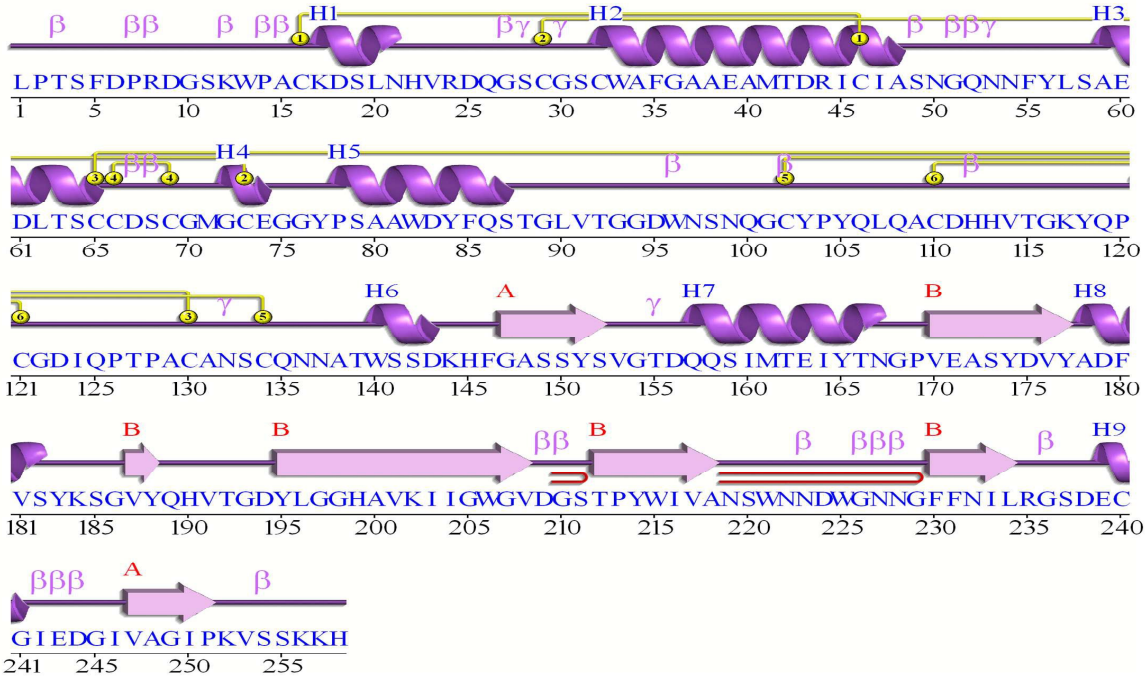

Fig. 1. Structural information obtained by PDBsum server for predicted model of cathepsin B from *Hordeum vulgare*. The letter H (blue) is for Helices, Strands are shown by A and B (red), beta and gamma turns represented by β and γ respectively. Yellow circles show disulphide bonds between residues.

Fig. 2. Sequence alignment between Cathepsin B from *Hordeum vulgare* and template (2IPP) Catalytic residues are (Red) indicated by arrows.

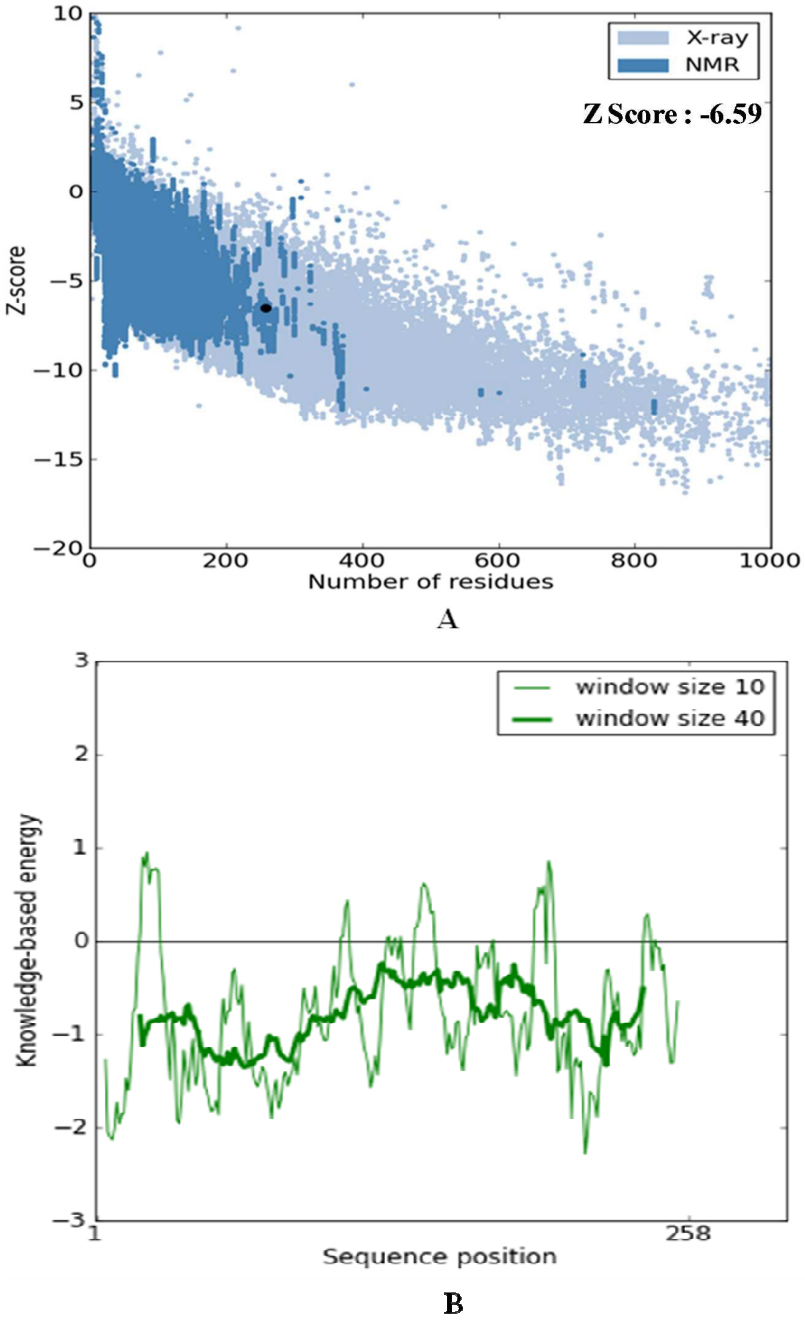

Fig. 3. The structure validation of homology model of Cathepsin B from *Hordeum vulgare* through ProSA.

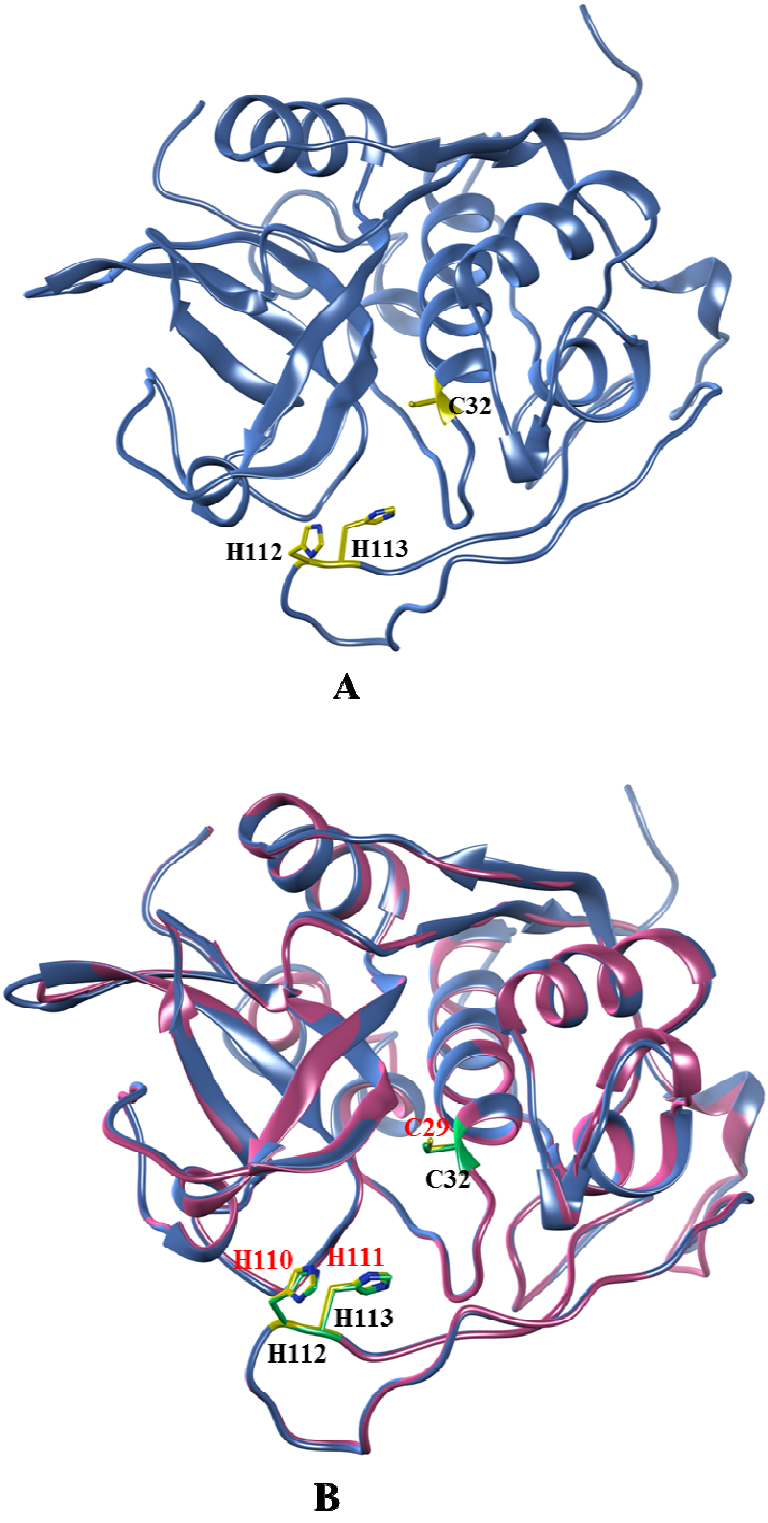

Fig. 4. A) Active site residues (Yellow) of plant cathepsin B **B)** Comparison of active site residues (Yellow) of cathepsin B from *Hordeum vulgare* (Cornflower blue) and active site residues (Green) human cathepsin B (Hot pink) (2IPP.pdb).

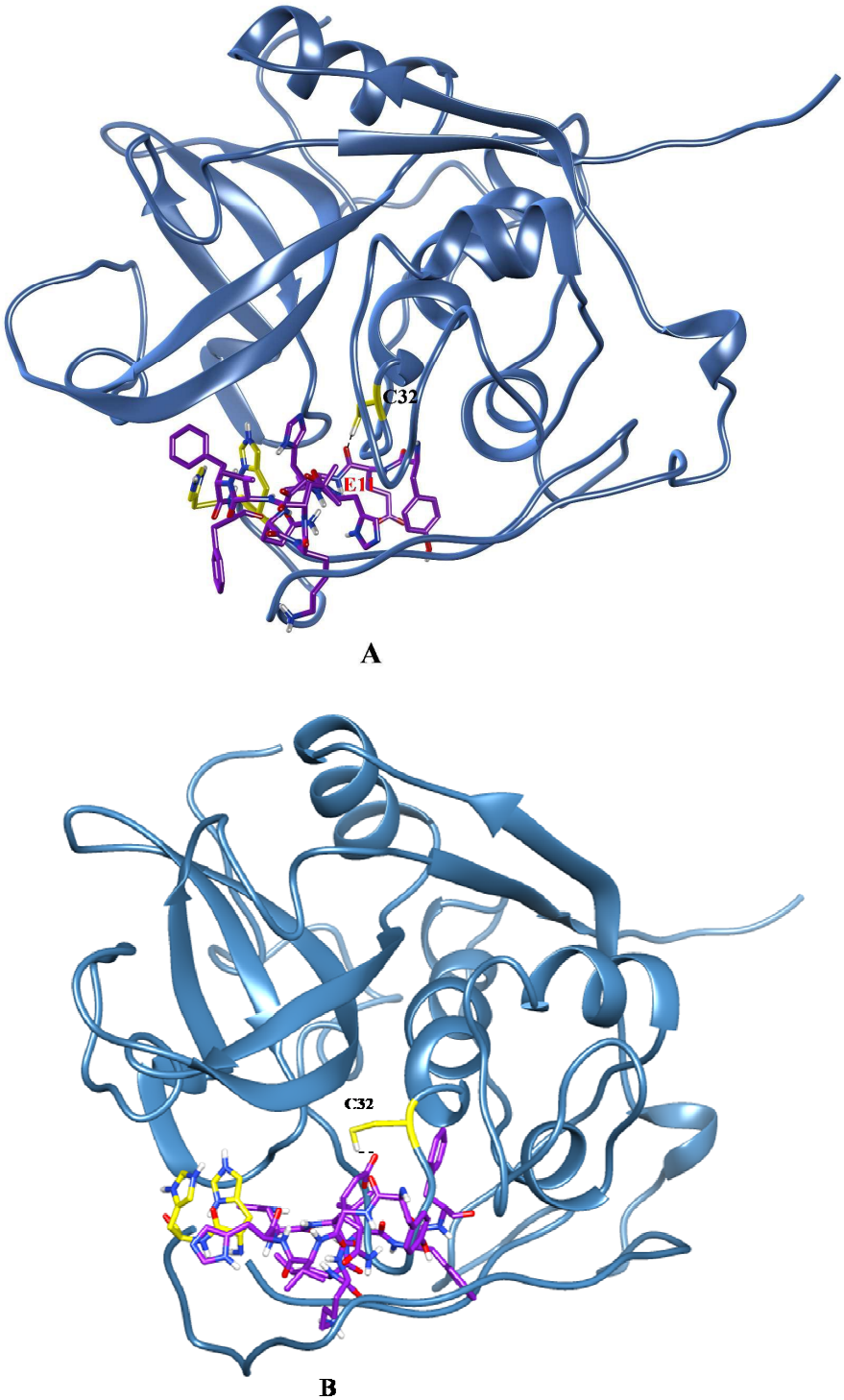

Fig. 5. Docked complex of Cathepsin B from *Hordeum vulgare* and patch of Aβ peptide **A)** Before MD **B)** After MD.

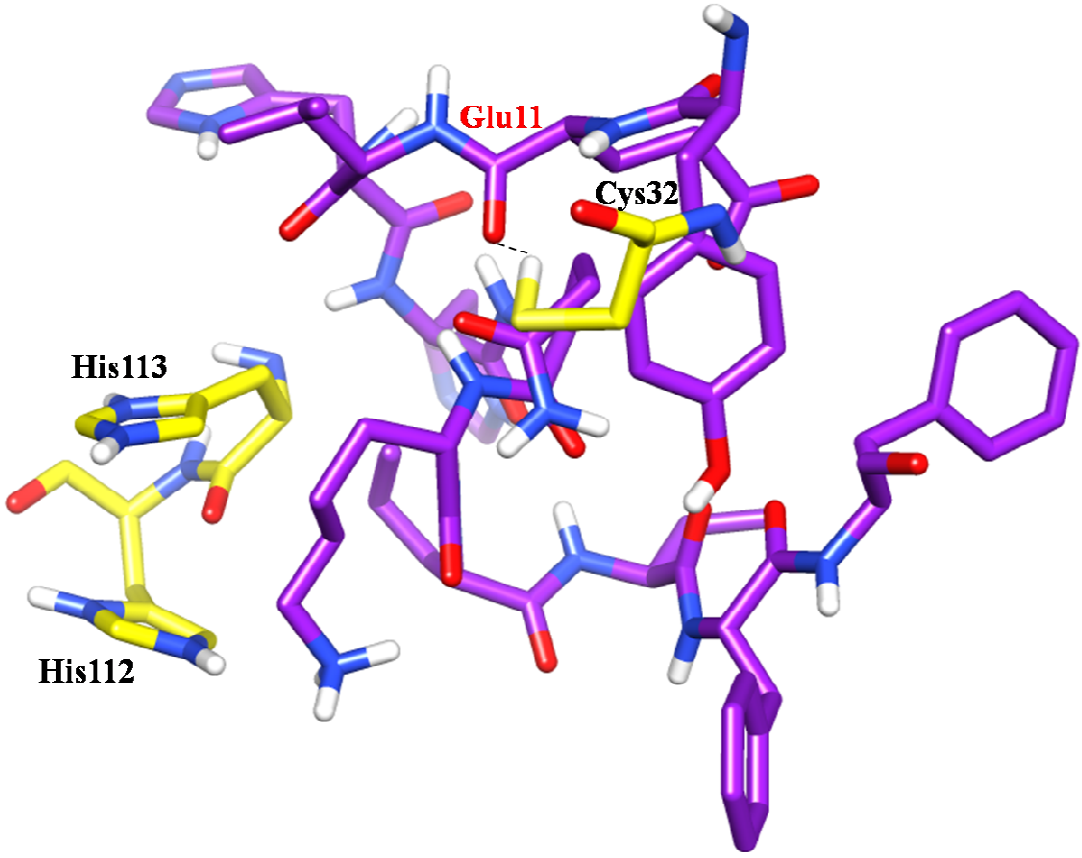

Fig. 6. Docked complex of Cathepsin B from *Hordeum vulgares* showing interaction between active site residues (Yellow) with Aβ peptide (Purple) after MD.

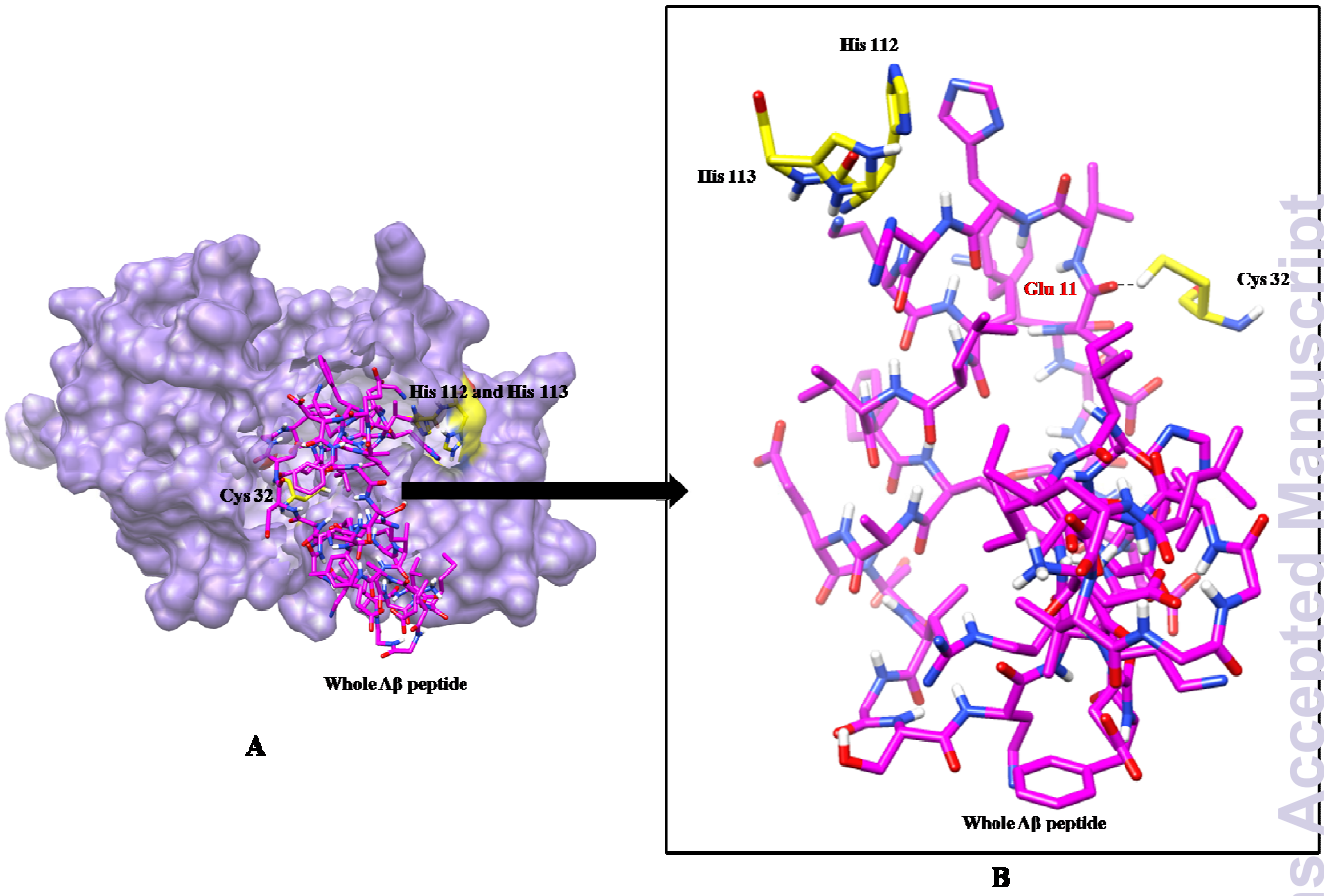

Fig. 7. A) Docked complex of Cathepsin B from from *Hordeum vulgare* (cornflower blue) with whole Aβ peptide (sticks in magenta color) (1AML.pdb). **B)** Interactions between active site residues (yellow) of Cathepsin B from *Hordeum vulgare* with whole Aβ peptide (magenta).

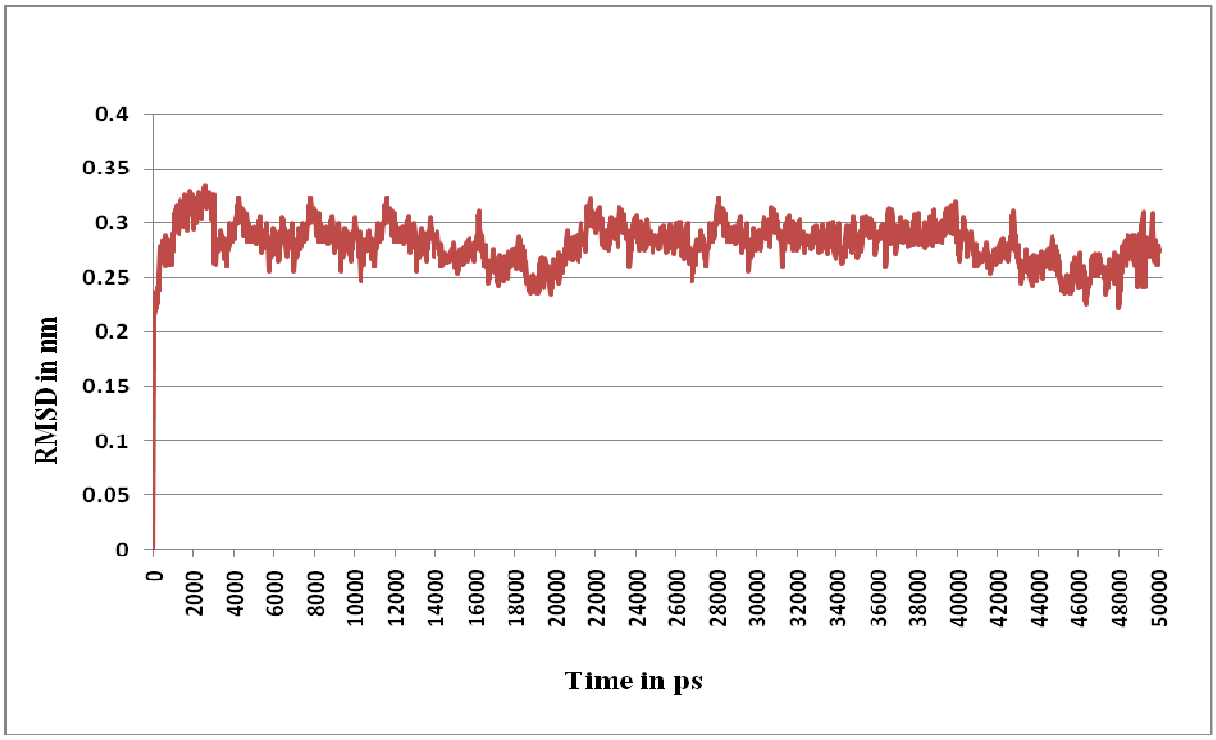

Fig. 8. RMSD of Docked complex of cathepsin B from *Hordeum vulgares* and Aβ peptide.

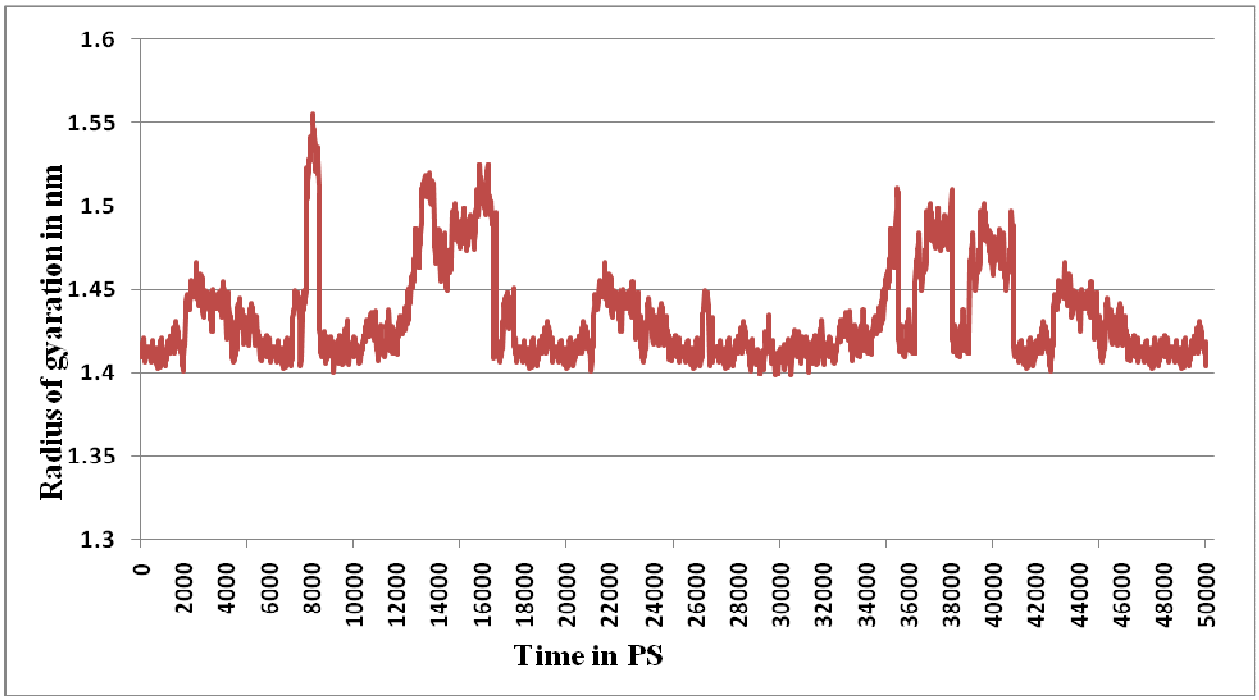

Fig. 9. Radius of gyration for Docked complex of cathepsin B from *Hordeum vulgares* and Aβ peptide.

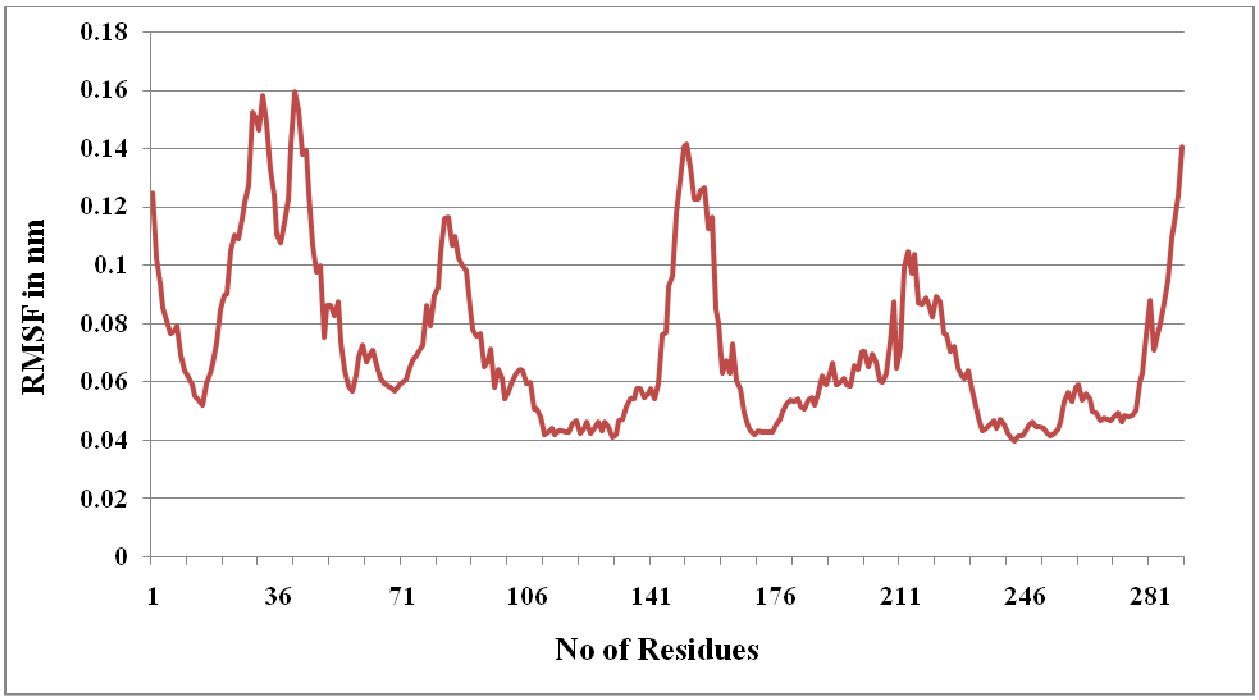

Fig. 10. RMSF of Docked complex of cathepsin B from *Hordeum vulgares* and Aβ peptide.

Table no 1. The PROCHECK analysis for models of plant cathepsin B obtained by Geno3D.

Model no.	Residues in core region	Residues in allowed region	Residues in generously region	Residues in disallowed region
Model 1	65.5%	27.4%	4.2%	3.0%
Model 2	63.1%	31.5%	4.2%	1.2%
Model 3	65.5%	30.4%	3.6%	0.6%
Model 4	65.5%	29.8%	3.0%	1.8%
Model 5	62.5%	31.5%	4.8%	1.2%
Swiss Model	77.6%	21.2%	0.6%	0.6%
Modeler	87.9%	10.7%	0.0%	1.4%

Table 2. Interactions between active site residues of Cathepsin B from *Hordeum vulgare* with patch of Aβ peptide ($A\beta_{10-20}$) before and after MD simulation.

Interaction between Aβ peptide and Active site residues Cathepsin B from *Hordeum vulgare* before MD.	**Atomic distance in Å**
GLU 11. O ------ CYS 32. HG:	2.503
Interaction between Aβ peptide and Active site residues Cathepsin B from *Hordeum vulgare* after MD.	**Atomic distance in Å**
GLU 11. O ------ CYS 32. HG:	2.003
GLU 11. H: ------ CYS 32. HG	2.428

Table 3. Interactions between active site residues of Cathepsin B from *Hordeum vulgare* with whole Aβ peptide ($A\beta_{1-40}$).

Interaction between whole Aβ peptide and Active site residues Cathepsin B.	**Atomic distance in Å**
TYR 10 O ------ CYS 32 HN2:	2.549
GLU 11 O ------ CYS 32 HN2:	2.697
CYS 32 HN2 ------ GLU 11 H:	2.949
CYS 32 HN2 ------ VAL 12 H:	2.176