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Homology modeling, molecular docking and MD simulation studies to investigate role of cysteine protease from *Xanthomonas campestris* in degradation of Aβ peptide

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ABSTRACT

Cysteine protease is known to degrade amyloid beta peptide which is a causative agent of Alzheimer's disease. This cleavage mechanism has not been studied in detail at the atomic level. Hence, a three-dimensional structure of cysteine protease from *Xanthomonas campestris* was constructed by homology modeling using Geno3D, SWISS-MODEL, and MODELLER 9v7. All the predicted models were analyzed by PROCHECK and PROSA. Three-dimensional model of cysteine protease built by MODELLER 9v7 shows similarity with human cathepsin B crystal structure. This model was then used further for docking and simulation studies. The molecular docking study revealed that Cys17, His87, and Gln88 residues of cysteine protease form an active site pocket similar to human cathepsin B. Then the docked complex was refined by molecular dynamic simulation to confirm its stable behavior over the entire simulation period. The molecular docking and MD simulation studies showed that the sulfhydryl hydrogen atom of Cys17 of cysteine protease interacts with carboxylic oxygen of Lys16 of Aβ peptide indicating the cleavage site. Thus, the cysteine protease model from *X. campestris* having similarity with human cathepsin B crystal structure may be used as an alternate approach to cleave Aβ peptide a causative agent of Alzheimer's disease.

1. Introduction

Cathepsin B is a cysteine protease which carries out a variety of physiological and pathological processes [1–3]. It degrades proteins which are entering endolysosomal system [4]. Amyloid plaque formation is the pathological hallmark in the Alzheimer's disease [5,6]. The Aβ (1–42) peptides are the main constituents of amyloid plaques in Alzheimer's disease [7–9]. The familial autosomal dominant (FAD) mutations and improper proteolytic degradation increase Aβ (1–42) peptides deposition [10]. The C-terminal truncation of Aβ (1–42) peptides by cathepsin B reduces amyloid beta peptide deposition. Hence, the enhanced activity of endogenous cathepsin B may decrease Aβ (1–42) peptide level in AD [11–14]. The reduced level of endogenous inhibitor cystatin C has been found useful to increase cathepsin B activity [15]. The enzymes such as IDE, NEP, ECE, ACE, MMP-9, and plasmin have a role in the Aβ peptide clearance [16–21]. The abnormal distribution of cathepsin B and its endogenous inhibitor leads to different neurodegenerative pathologies [22]. The reduced cathepsin B immunoreactivity was observed due to increase in its inhibitor concentration that leads to the Aβ peptide deposition [23]. Therefore, low levels of endogenous cathepsin B inhibitors (secretory cystatin C and cystatin B) may increase cathepsin B activity and clearance of amyloid plaques [24].

There are various enzymes in human brains that decrease the load of Aβ peptides but the factors such as enzymatic loss through genetic mutations or nongenetic reasons like direct oxidative damage or enhanced production of inhibitors may result in to abnormal Aβ catabolism [25]. Thus, further studies to search enzymes from novel bacterial sources having potential to degrade Aβ peptides are essential. The amyloid-degrading ability of Nattokinase enzyme from *Bacillus subtilis* Natto has been reported earlier [26]. Similarly, aminopeptidase from *Streptomyces griseus* K565 also showed Aβ peptide degradation activity [27] which suggests that the bacterial enzymes may be used for Aβ peptide clearance. The present work has been carried out to investigate the role of bacterial enzymes to degrade Aβ peptide that may be further used for drug development studies in AD research.

Abbreviations: AD, Alzheimer's disease; NEP, neprilysin; IDE, insulin degrading enzyme; ACE, angiotensin converting enzyme; ECE, endothelin converting enzyme; MMP-9, matrix metalloprotease-9; Aβ peptide, amyloid beta peptide; MD, molecular dynamics; RMSD, root mean square deviation

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The three-dimensional model of the cysteine protease from a bacterial source *Xanthomonas campestris* was developed by using MODELLER 9v7 software. The steepest descent energy minimization method was used to remove steric clashes. The structure was validated through various online servers which confirm the structure quality. The sequence analysis and model comparison between cysteine protease from *X. campestris* and human cathepsin B showed good homology and resemblance in the active site pocket. Superimposition of predicted structure of cysteine protease and human cathepsin B [28] showed a similar type of active site residues. Experimental results showed that the human cathepsin B cleaves Aβ peptide at Lys16 [29]. Our molecular docking study revealed that the active site residue Cys17 of cysteine protease from *X. campestris* interacts with Lys16 of Aβ peptide hence it may cleave Aβ peptide from carboxylic site of Lys16 similar to human cathepsin B [29]. The molecular dynamic simulation of model and Aβ peptide complex verified stability of the docked complex. Therefore, the predicted cysteine protease model from *X. campestris* could be used to study Aβ peptide degradation activity which might be helpful to develop a new therapeutic approach for the AD patient's treatment.

2. Methods

2.1. Software and hardware

In order to get a good model of cysteine protease homology modeling was done by using SWISS-MODEL [30], Geno3D [31] and MODELLER 9v7 [32]. The predicted models were evaluated by various online servers such as PROCHECK [33] for the Ramachandran plot quality evaluation, PROSA [34] for testing interaction energies and VERIFY-3D [35] for measuring the compatibility. A molecular docking study was carried out between the best model of cysteine protease and Aβ peptide using AutoDock 4.2 [36]. The docked complex was then used for the MD simulation study. All the docking and simulation studies were done on HP workstation and Rack/Blade server. Interactive visualization and analysis of molecular structures were carried out using chimera [37] and VMD [38].

2.2. Sequence alignment and homology modeling

The complete amino acid sequence of the cysteine protease from *X. campestris* was retrieved from NCBI protein sequence database (Accession no.—ZP_06488281.1). The BLAST program was used to search suitable template available in the PDB. The crystal structure of human cathepsin B (PDB ID: 2IPP) [28] with resolution of 2.15 Å was used as a template to build a cysteine protease model. The pairwise sequence alignment of the template sequence (2IPP) and the cysteine protease sequence was done using the EMBOSS program [39].

Homology modeling of the cysteine protease was performed by three homology modeling programs such as SWISS-MODEL [30], Geno3D [31] and MODELLER 9v7 [32]. For model building the alignment between template and query sequence was done by using global dynamic programming with linear gap penalty function available in MODELLER 9v7 [32]. The model was constructed by satisfaction of spatial restraints, using its 'automodel' class. The model was generated using the python script command. The steepest descent energy minimization was done to remove steric clashes [40].

2.3. Secondary structure analysis, model refinement and validation

Secondary structure analysis of the cysteine protease sequence was done by Self-Optimized Prediction Method (SOPMA) Program [41]. The models obtained by SWISS-MODEL [30], Geno3D [31] and MODELLER 9v7 [32] were validated by inspection of the Phi/Psi Ramachandran plot [42] obtained from PROCHECK analysis [33]. The model constructed by MODELLER 9v7 [32] was finally chosen for further investigations on the basis of geometry, 3D alignment with the template and the results of PROCHECK [33] and PROSA [34] analyses. The PROSA [34] test was applied to check the energy criteria of predicted models in comparison with known X-ray and NMR structures. The PROSA II energy plot was calculated to check the interaction energies of all residues of the predicted model. Further, the model quality was evaluated by VERIFY-3D [35]. The VERIFY-3D [35] compares model with its template structure in terms of three-dimensional and sequence score (3D–1D). The Ramachandran plot was obtained through PROCHECK analysis [33]. The predicted model was then compared with a template structure (PDB ID: 2IPP) [28] by using the PDBeFOLD server [43] and chimera [37].

2.4. Active site prediction

The active site prediction in the refined model was done by sequence alignment. The sequences of cysteine protease from *X. campestris* was aligned with sequence of human cathepsin B (PDB ID: 2IPP) [28] for which the active site is known. The 3D alignment of cysteine protease and human cathepsin B (PDB ID: 2IPP) was done by chimera [37] to compare the active site pocket.

2.5. Molecular docking of cysteine protease and Aβ peptide

Molecular docking has been performed between cysteine protease model and patch of Aβ peptide (^{10}YDVHHNKLVFF^{20}) from 1AML.pdb [44] by using AutoDock 4.2. [36]. The docked complex was then minimized by the steepest descent (SD) method using Gromacs 4.0.4 to remove hard contacts [40]. The Lamarckian genetic algorithm (LGA) has been used in this study. The active site was defined using Auto Grid [36]. The grid size was set to $40 \times 44 \times 46$ points with a grid spacing of 0.375 Å centered on the selected flexible residues present in the active site of cysteine protease. The grid box has the entire binding site of the cysteine protease and provides enough space to the Aβ peptide for the translation and rotation. Step size of 2 Å for translation and 50° for rotation were chosen and the maximum number of energy evaluation was set to 25,00,000. Thus, 10 runs were performed and for every independent run a maximum number of 27,000 GA operations were generated on a single population of 150 individuals. Finally, docked complex with lower binding energy has been selected for further analysis.

2.6. Molecular dynamic simulation of predicted model

Molecular dynamic simulation was performed by Gromacs 4.0.4 program [40] by using OPLS-AA force field [45,46]. The complex of cysteine protease and patch of Aβ peptide ^{10}YDVHHNKLVFF^{20} was used for MD simulation. The complex was surrounded by SPC water molecules (water density 1.0) and system was neutralized with three Na^+ ions. The cubic type of box was used for MD simulation. The MD simulation system has a total of 41,600 atoms. The solvated structure was minimized by the steepest descent method for 15,000 steps at 300 K temperature and constant pressure. Then the complex was equilibrated for 2 ns period. After equilibration production MD was run for 20 ns at constant temperature and pressure. The LINCS algorithm [47] was used to constrain the bond length. Periodic boundary conditions were applied to the system. The electrostatics interactions were calculated using a PME algorithm with 12 Å cut off [48]. Structural comparison between initial structure and final structure of MD simulation was done using PDBeFOLD [43]. The dynamic runs