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Molecular Modelling Studies Of Keratinase From *Streptomyces Badius Strain Shashi*: An *In-Silico* Approach To Understand The Keratin Hydrolysis

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Abstract:

There are many enzymes produced by microorganisms having potential applications in industrial sectors. Out of which keratinase is one of the major enzymes having applications in tanning industry. The exact role of keratinase in cleaving keratin substrate has not been well studied. In present study, an attempt is made to understand the amino acid residues of keratinase interacting with keratin. The predicted model of keratinase was validated by Ramchandran plot and Verify-3D to check the quality of model. The docked complex of keratinase with keratin revealed the stable hydrogen bonding interactions. After molecular dynamics (MD) simulation studies it has observed that the amino acid residues such as PRO74, VAL158 and THR262 of keratinase from *Streptomyces badius strain shashi* showed stable hydrogen bonding interactions with GLU390, GLN393 GLU394, ASN395, TYR398 of keratin substrate. Hence, MD analysis supports that keratinase from *Streptomyces badius strain shashi*. can efficiently cleave keratin. Thus, the present study would be useful to understand the enzymatic mechanism of keratinase to cleave keratin in detail at molecular level.

Keywords: *Streptomyces badius strain shashi,* keratinase, Homology modelling, Ramchandran plot, Molecular Dynamic Simulation.

Introduction

The outer layer of human skin possesses an important protein namely keratin (Navarro et al., 1995). This keratin is also occurred in hairs, wool, horn and nails. The keratin is a structural protein formed by assembly of monomers generated into intermediate filaments. The keratin is highly stable and it has low degradation rate due to presence of high degree of hydrogen bonds, disulphide bridges, and hydrophobic interactions (Bradbury 1973). The tightly packed helix and sheet gives stability to keratin structure (Kreplak et al. 2004). The disulphide bonds give stability and resistant against proteolytic degradation to the keratin. Keratin is divided into two families based on its sulphur content. one is hard keratin which includes nail, hoof, horn, hair and feather; another is soft keratin which includes skin (Lynch et al., 1986). The 7.3 cysteine per 100 residues are present which indicates the high mechanical strength of keratin. However, the keratin can be hydrolysed by an enzyme called keratinase (Bhari et al., 2019). These keratinases are proteolytic in nature and classified as proteinase with EC number 3.4.11.25 (Riffel et al. 2007). The peptide and disulphide bonds present in the keratin are hydrolysed by the keratinase. Keratinase are found in different sources such as archaea, fungi and bacteria species(Gopinath et al., 2015). The thermostable enzymes from hyperthermophiles and thermophiles are named as thermozymes, which possess remarkable features like activity at high temperature, heat stable and resistant to denaturation conditions by detergents and solvents (Bhari et al., 2019). In presence of detergent Sodium Dodecyl Sulphate (SDS) the thermostable keratinase showed good activity. This thermostable nature of keratinase found useful in industries where high temperature conditions may present. The high temperature in any process has advantages like less chance of bacterial contamination, reduces mixing speed, lower viscosity, improve transfer rate and increasing substrate solubility (Vetriani et al. 1998).

It is well known that keratinase has important application in different industries but still their enzymatic mechanism is unknown. Also, the production and optimization of keratinase activity still needs to be enhanced in order to make their industrial use feasible. Thus, the aim of present investigation was to predict the three-dimensional structure of keratinase from *Streptomyces badius. strain shashi* Hence, a homology model of keratinase from *Streptomyces badius.strain shashi* was predicted successfully and further it was validated by using online servers like Ramchandran plot analysis and Verify-3D that showed the good quality of predicted model. Also, molecular dynamic simulation studies of keratinase from *Streptomyces badius.strain shashi* was carried out to know the structural stability, which revealed stable hydrogen bonding interactions with keratin. Hence, this predicted structure of keratinase from *Streptomyces badius.strain shashi* found useful to understand the mechanism of enzymatic reaction to degrade keratin.

Material and methods

Sequence retrieval and homology modelling of Keratinase enzyme

The protein sequence of Keratinase was extracted from NCBI protein sequence database. The Keratinase sequence was then subjected to homology modelling studies to build the three-dimensional structure by MODELLER software (Sali and Blundell 1993). The predicted Keratinase structure with a good DOPE score was further used for Molecular Docking and molecular dynamic (MD) simulation studies.

Secondary Structural Analysis of Keratinase from *Streptomyces badius strain shashi*

Ramachandran plot showed allowed regions of phi-psi torsion angles for all residues in the structure (Ramachandran et. al., 1963). Glycine residues were separately identified by triangles. The colouring/shading on the plot represents different regions; the red areas correspond to the "core" regions, representing favourable combinations of phi-psi values. The percentage of residues in the "core" region is one of the better guides to stereochemical quality. Also, residues in the disallowed region should ideally be less than or equal to 0.2%.

Molecular docking of predicted structure of Keratinase with Keratin

Molecular docking is a technique to find out interactions between drug and receptor as well as protein and its activator/inhibitor (Dhanavade et al., 2013; Jalkute et al., 2013; Parulekar et al., 2013). A patch of keratin substrate having residues EMEQQNQEYKILLDV has been considered in this study. PATCHDOCK has been used to find out the mode of action of predicted structure of Keratinase with Keratin (Axenopoulos et al., 2013).

Molecular dynamic studies of complexes

Molecular modelling studies such as molecular dynamic simulation (MD) found very useful for the prediction of proper interactions between enzyme and its ligand (Barale et al., 2019; Dhanavade and Sonawane 2014; Dhanavade et al., 2013; Dhanavade et al., 2016). In present study, we have carried out MD of Keratinase model for 20ns. Then simulated model of Keratinase was docked with the Keratin substrate using PATCHDOCK (Axenopoulos et al., 2013). Further, the MD simulation of docked complex of keratinase with keratin substrate was carried out by using GROMACS version 5.1.2 with standard GROMOS96 43a1force field (Spoel et al., 2005; Abraham et al., 2015). The docked complex was solvated by using single point charge (SPC216) water model. The total charge in system was neutralized and then steepest descent method was used to minimize the solvated structure at 300 K temperature and constant pressure to remove steric clashes in docked complex (Spoel et al., 2005). The periodic boundary condition (PBC) was applied in all directions, followed by 50000 steps of steepest descent energy minimization. The particle mesh Ewald (PME) was used to calculate short-range and long-range electrostatic interactions (Essmann et al., 1995). The Linear Constraint Solver algorithm was used to constrain all bonds (Hess 2008). After minimization, the system of docked complex was equilibrated under NVT (constant number

of particles, volume, and temperature) condition for 500 ps at 300 K followed by another 500 ps run under NPT (constant number of particles, pressure, and temperature) at 300 K applying position restraints. Then production MD of the equilibrated complex was run for 20ns. After completion of MD, the docked complex was analysed and images were built by using structure visualization software such as CHIMERA (Pettersen et al., 2004).

Binding Free Energy Calculation

The relative binding energy of protein-ligand complex is widely used in MD simulations.MM-PBSA in combination with MD simulations is used to calculate the binding energy of protein and ligand complexes using the equation **Δ** G(Binding) = G (Complex) – G (Receptor) -G- (Ligand), where G (Complex) is total free energy of the ligand-protein complex, G (Receptor) and G (Ligand) are total free energies of the isolated protein and ligand in solvent, respectively. G-mmpbsa -A GROMACS tool for high-throughput MM-PBSA calculations (Kumari et al., 2014)

Results and Discussion

Homology modelling of Keratinase from *Streptomyces badius*

The three-dimensional structure of Keratinase from *Streptomyces badius.strain shashi* was built by using MODELER (Sali and Blundell, 1993) (Figure 1). The DOPE score revealed that all of the models of Keratinase from *Streptomyces badius strain shashi* generated by MODELLER were of good quality. The best three-dimensional structure of Keratinase was further selected for docking and molecular dynamic simulation studies. After Molecular Dynamic Simulation, the docked complex was further analysed by using UCSF CHIMERA software (Pettersen et al. 2004) for hydrogen and other interactions.

Secondary structural analysis of Keratinase from *Streptomyces badius strain shashi*

The protein model generated by homology modelling was further analysed from the Ramachandran plot (Laskowski et al., 2018, Laskowski et. al., 1993), showed only 2 amino acids in the disallowed region and a total of 99.12% residues in the allowed regions, hence showed good quality (Figures 2). Similarly, the Verify-3D analysis revealed that the predicted model has Z-Score -7.06, which is in accordance with the standard structures of X-ray and NMR studies. Verify3D analysis revealed that 83.52% of the residues have averaged 3D-1D score >= 0.2 (Figure 2). These results indicating that predicted model having good quality 3D structure.

Molecular dynamic simulation of Keratinase enzyme

The predicted model with good quality was further analysed by performing 20ns MD and the structural changes between models before and after MD was checked by superimposing them using UCSF chimera tool (Figure 3).

Molecular dynamic simulation of Keratinase enzyme complexed with keratin as a substrate.

The MD simulation of Keratinase model was carried out which showed that it has 0.33 nm root means square deviation (RMSD) that shows the stability of the keratinase model (Figure 4). Further, the simulated Keratinase model was docked with Keratin substrate and then this complex was subjected to fully solvated MD simulation. After the MD simulation, the docked complex of keratinase with keratin substrate was analysed to check its stability during MD simulation. The RMSD of the docked complex after MD simulation was 0.20 nm (Figure 4). Hence from this study, it is clear that the keratinase complexed with keratin substrate has more stability than only keratinase structure. Further, it has been confirmed by analysing the root means square fluctuations (RMSF) of Keratinase 0.12 nm and the docked complex which was found to be 0.10 nm (Figure 5).

Hence, these RMSD (Figure 4) and RMSF (Figure 5) results strongly support that the docked complex is stable. The docked complex after molecular dynamic simulation was analysed by using structure visualization software such as CHIMERA (Pettersen et al., 2004). Thus, the MD analysis revealed that this complex is stable.

Analysis of Docked Complex after Molecular Dynamic Simulation

The analysis of docked complex showed that there are strong hydrogen bonding interactions between predicted and simulated structure of Keratinase enzyme with keratin as a substrate. There are many hydrogen bonding interactions observed between the active site residues of keratinase like PRO74, VAL158, and THR262 with keratin residues such as GLU390, GLN393 GLU394, ASN395, TYR398. These interactions found to be stable after the 20ns molecular dynamic simulation (Figure 7 and Table 1).

Binding free energy Calculation

The binding free energy for the keratinase-keratin complex is shown in the table 2. The estimated binding free energy for the complex is -570.142 +/- 40.398 kJ/mol.

Concerted motion during the MD simulation

Dynamic cross correlation matrix has been analysed to gain detailed insights to the concerted motion exerted by various flexible regions of the Keratinase. A comparative analysis on the domain motions of free and keratin bound keratinase has been performed to understand local conformational changes at structural level in the keratinase upon keratin binding. Figure 8 depicts the cross-correlation motion observed with free keratin (Panel A) and keratin bound keratinase (Panel B). The diagonal line (amber colour) expresses the strong autocorrelation between keratinase residues. The amplitude of correlated to anticorrelated motions is scaled from amber to blue, respectively. In free keratinase, N and C-terminal domain expresses moderate to strong negative correlation with each other, whereas, C-terminal domain show positive correlation with itself. Whereas keratin bound keratinase showed relatively much stronger positive correlation at all the regions except flexible loop region formed by residues

Conclusion:

In the present study, a three-dimensional structure for keratinase from *Streptomyces badius.strain shashi* was predicted. Ramchandran Plot and Verify-3D analysis the predicted model of keratinase showed good quality. Further, molecular docking studies revealed that keratinase enzyme bound with keratin with forming several hydrogen bonding interactions with active site residues such as PRO74, VAL158, and THR262. MD results revealed stable hydrogen bonding interactions between keratinase-keratin complex throughout the 20ns MD simulation period. It is well supported by RMSD, RMSF and Rg graphs. Hence, MD simulation analysis supports that keratinase from *Streptomyces badius.strain shashi* can efficiently cleave keratin substrate. Thus, the present computational study could be useful to understand the enzymatic mechanism of keratinase to cleave keratin at molecular level.

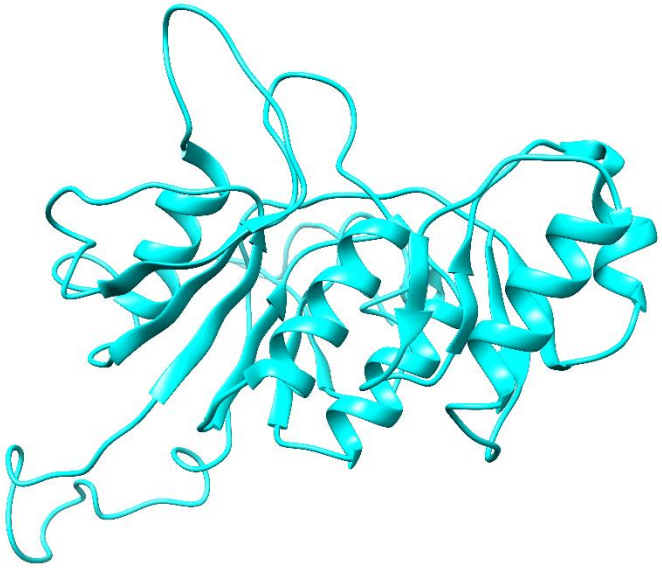

Figure 1: Predicted Model of Keratinase enzyme model after Homology Modelling by Modeller

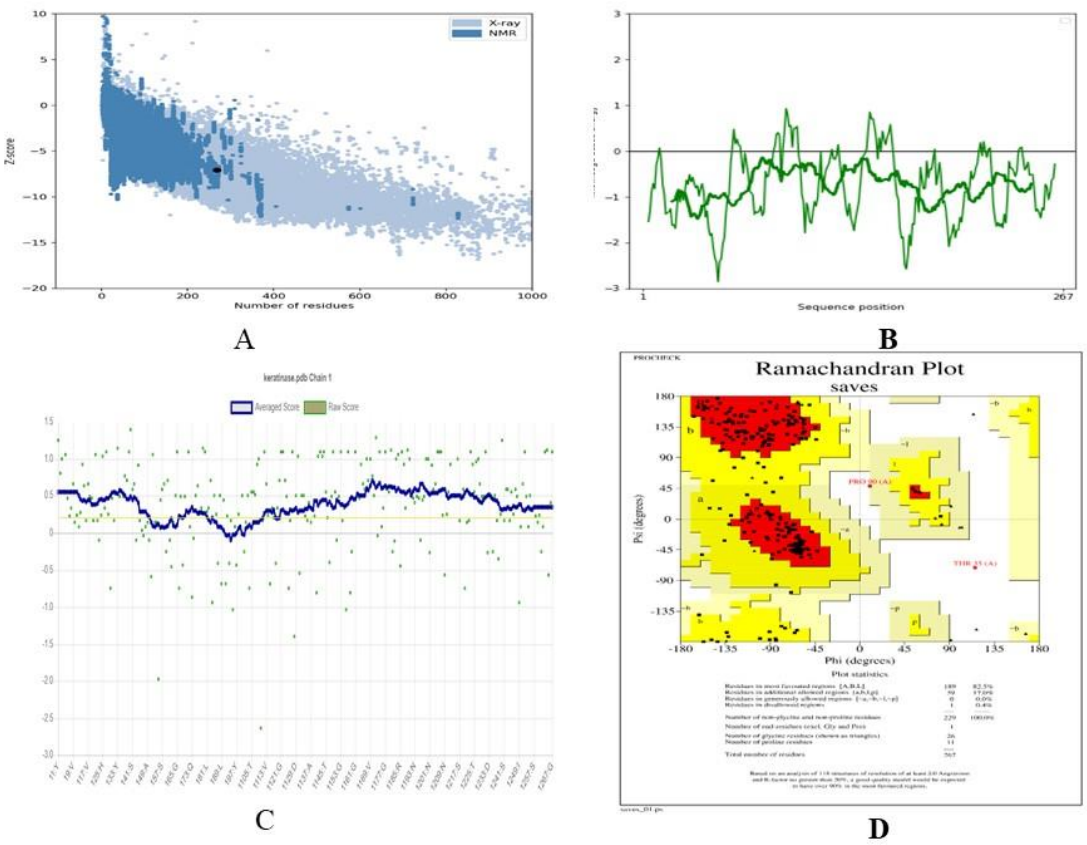

Figure 2: PROSA analysis of Keratinase model A) Z Score, B) Local model quality, C) PROSA analysis, D) Ramachandran plot of Keratinase model.

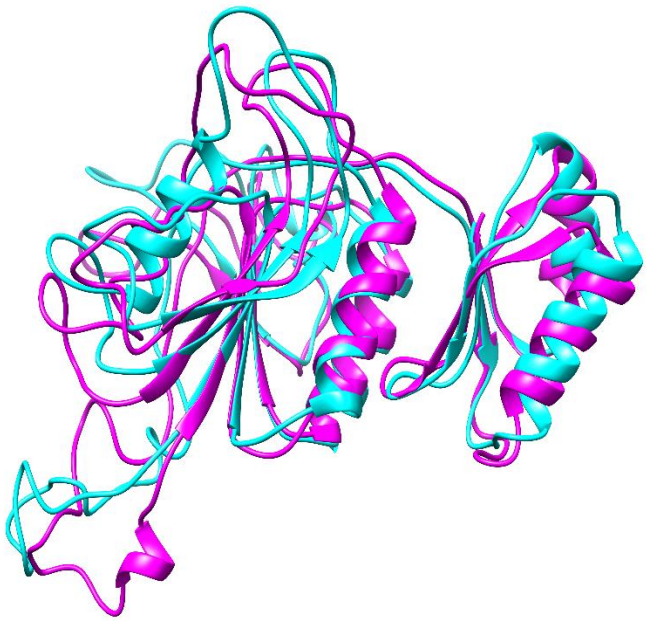

Figure 3: Superimposition of Keratinase Model Before MD(Cyan) and after MD (Magenta)

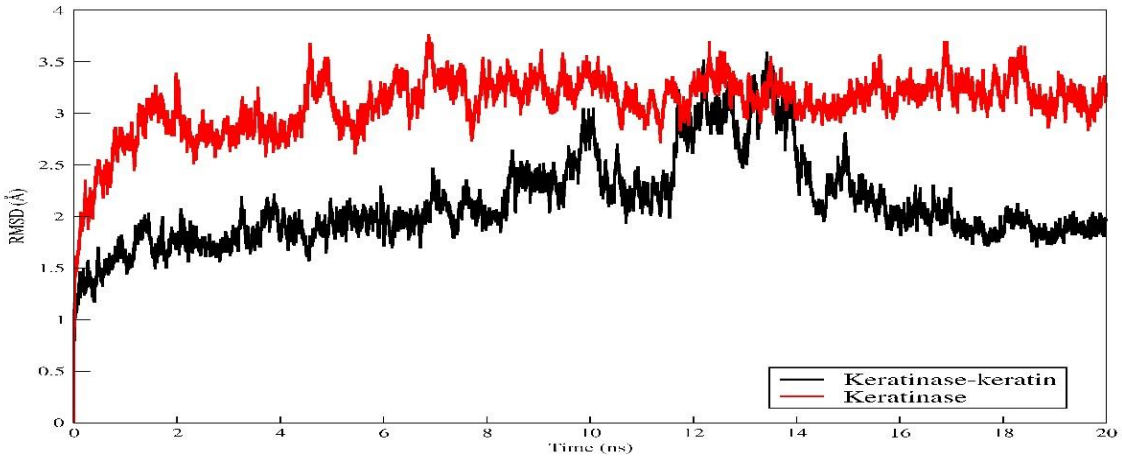

Figure 4: RMSD of predicted Keratinase model (Red) and Docked complex of Keratinase with Keratin (Black) after 20ns MDS.

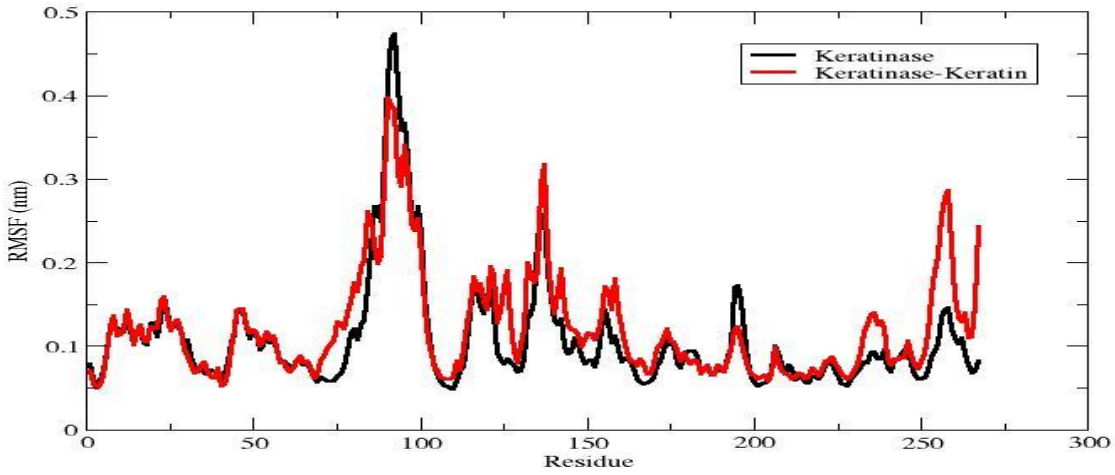

Figure 5: RMSF of Keratinase (Red) and Docked complex of Keratinase with keratin (Black) after 20ns MDS.

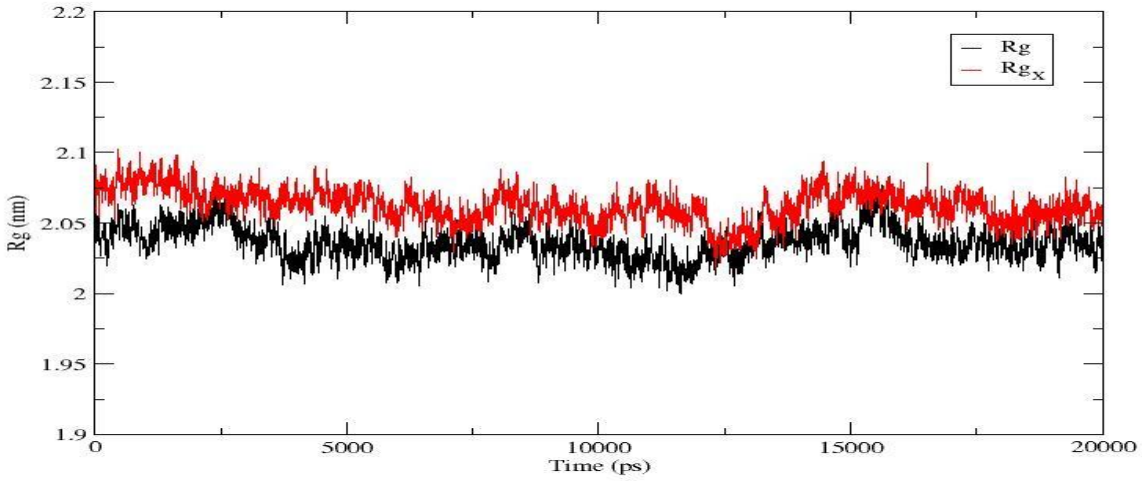

Figure 6: Radius of Gyration (Rg) of Keratinase (Black) and Docked complex of Keratinase with keratin (Red) after 20ns MDS.

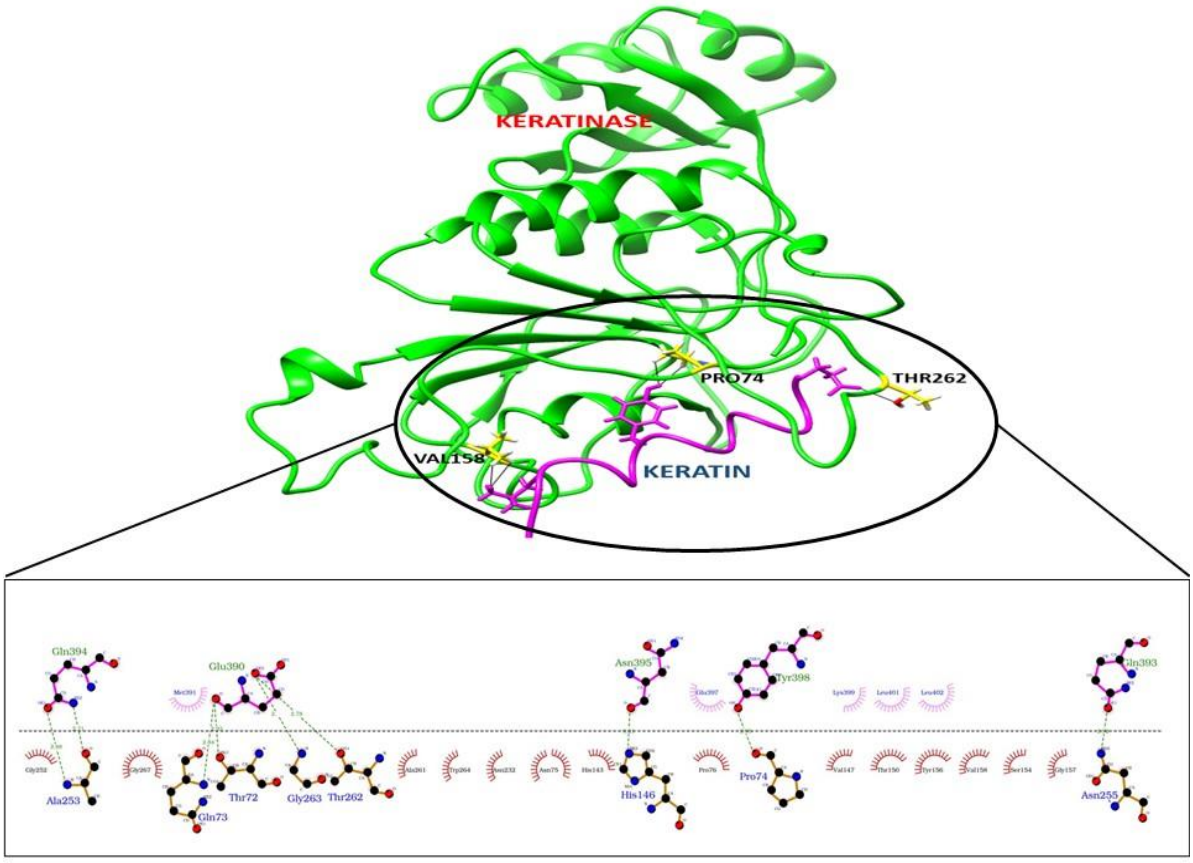

Figure 7: Docking interaction of active site residues (yellow) of Keratinase (Green) with Keratin (Magenta).

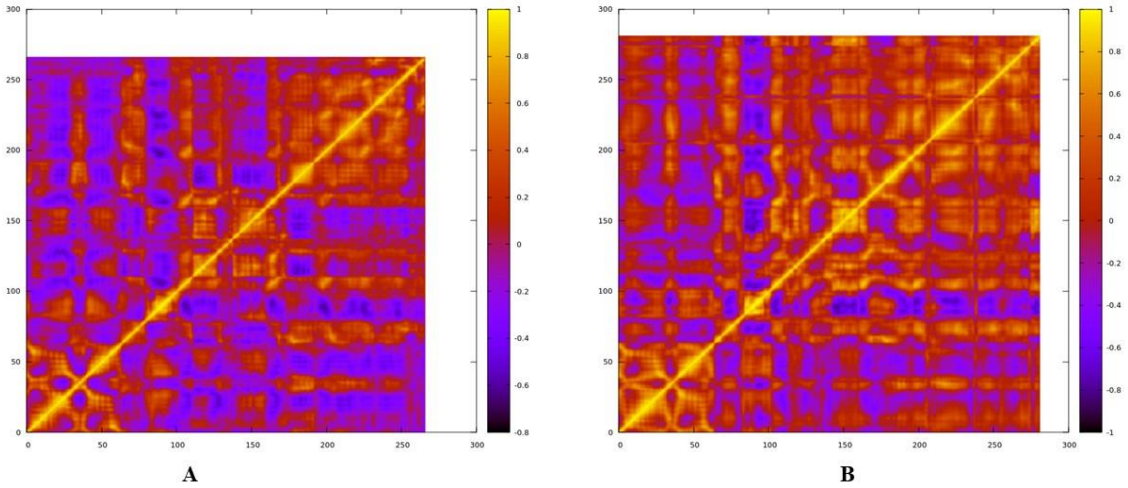

Figure 8: Concerted motion during the MD simulation A) Keratinase, B) Keratinase-Keratin Complex

Table 1: Hydrogen bonding interactions between keratinase and keratin after MD.

Sr. No.	**Interaction between active site residues of Keratinase with keratin.**	**Distance in Å**
1	THR 262 OG1 ------ GLU 390 OE2:	2.9
2	LEU 402 2HD1 ------ VAL 158 2HG1:	2.8
3	LEU 402 2HD1 ------ VAL 158 1HG1:	2.5
4	TYR 398 HH ------ PRO 74 HG1:	2.8
5	TYR 398 HH ------ PRO 74 HD1:	2.9

Table 2: summary of binding energies of Keratinase-Keratin complex after MD simulation

Complex	**Δ_{Evdw} kcal/mol**	**Δ_{Eelec} kcal/mol**	**Δ_{Gpolar} kcal/mol**	**$\Delta_{Gnon-polar}$ kcal/mol**	**$\Delta_{Gbinding}$ kcal/mol**
Keratinase-Keratin	-90.217 +/- 5.42	-103.517 +/- 8.74	-67.52 +/- 8 .28	-10.06 +/- 0.509	-136.26 +/-9.65

Table 3: Analysis of MD trajectories for RMSD, RMSF, Rg of Keratinase and Keratinase-keratin complex

Sr. No.	**MD Properties**	**Keratinase**	**Keratinase-keratin complex**
1	Root Mean Square Deviation (RMSD) (nm)	0.33	0.20
2	Root Mean Square Fluctuation (RMSF) (nm)	0.12	0.10
3	Radius of gyration (Rg)	2.03	2.06

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