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A challenging biostimulant used in agriculture: Papain – study on its mechano-chemical properties

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Abstract

Papain is a cysteine protease used in agriculture as a plant biostimulant, stimulating physiological processes in plants and enhancing crop yield. A plant biostimulant can be any substance or microorganism used on plants to increase nutrition efficiency and to improve its nutrients content. In our study, we have considered the complex structure of papain with CLIK148, which is an experimental model structure to understand the cathepsin L. inhibitors. The complex structure of papain with CLIK148 has been refined at 1.7 Å resolution. The mechano-chemical properties of papain molecule were investigated by molecular dynamics simulations using NAMD 2.13 software. We have performed both equilibrium/non-equilibrium and tensile tests on the complex structure of papain CLIK148. In conclusion, we have developed atomistic modelling of the papain structure gaining new insights into mechano-chemical properties (energy and temperature distribution, heat diffusion and steered molecular dynamics) of this cysteine protease which is used as a biostimulant in agriculture.

Keywords: papain, plant biostimulant, mechano-chemical properties, molecular dynamics simulations

1. Introduction

Biostimulants are various substances obtained from natural sources and have beneficial actions on plant growth and development, stress resistance and crop yield [1-3]. Natural plant biostimulants (PBs) are used to enhance flowering, plant growth, fruit quality [4], crop productivity and to improve the tolerance against a wide range of abiotic stresses [3,5]. Lately, researchers are focused on the non-microbial and microbial plant biostimulants, such as protein hydrolysates (PHs) which consist of a mixture of peptides and amino acids [1,3]. Proteases from plants used for PHs production are cysteine proteases (Cys residue at the catalytic site) such as chymotrypsin, papain and bromelain, which can be used as biostimulants in horticulture [1,6].

Papain-like cysteine proteases (PLCPs) is an essential enzyme, which is involved in plant development and multiple physiological processes [7,8].

Conformational changes and structural properties of papain were investigated using molecular dynamics simulations and semiempirical methods, respectively. Thus, the structure-activity relationship in the case of the papain hydrolysis of certain N-benzoylglycine esters was analyzed using molecular dynamics and full structure Local SCF semi-empirical quantum mechanics calculation of receptor-ligand complexes [9]. Docking and molecular dynamics simulations were used to study the substrate specificity of papain dynamic structures for peptides of 8-10 glycine residues (8-10GLY) [10] and the metastability of papain and the molecular mechanism for its sequential acid-denaturation [11].

Our main goal was to investigate the mechano-chemical properties of papain molecule using molecular dynamics simulations. In our study, we have considered the complex structure of papain with CLIK148, which is an experimental model structure to understand the cathepsin L. inhibitors

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[12]. Also, the papain/CLIK148 coordinate system is a model used to explore the interactions of a nonpeptide thiocarbazate inhibitor of cathepsin L. using docking simulations [13]. To investigate the mechano-chemical properties of papain/CLIK148 complex structure, we have performed both equilibrium/non-equilibrium analysis (energy and temperature distribution, heat diffusion) and tensile tests (steered molecular dynamics). In conclusion, we have developed an atomistic model of the papain structure to study the mechano-chemical properties of this cysteine protease, which is a challenging biostimulant used in agriculture.

2. Materials and Method

2.1. Simulation procedures

The mechano-chemical properties of papain molecule were investigated by molecular dynamics simulations using NAMD 2.13 software [14] with CHARMM force field for proteins and lipid [15] and VMD 1.9.3. The protein structure used in our simulations was papain/CLIK148 system, which is an experimental model structure to understand the cathepsin L. inhibitors. The complex structure of papain with CLIK148 has been refined at 1.7 Å resolution and was obtained from Protein Data Bank (PDB ID: 1CVZ) [12]. This crystal structure contains 212 residues (Figure 1).

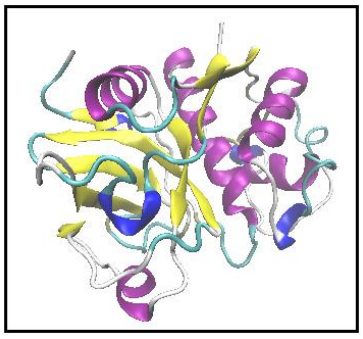

Figure 1. Papain/CLIK148 complex structure represented in VMD editor (PDB ID: 1CVZ) (five pieces of α-helix – mauve color, short pieces of 310-helix – blue color and a mixed β-sheet with seven strands – yellow color)

Our protein structure was minimized and equilibrated with periodic boundary conditions. The papain/CLIK148 complex structure of 61×57×53 $Å^3$ was placed into a water box of 64×60×54 $Å^3$ with a water layer of 5 Å, in which we utilized the TIP3P model for water molecules (Figure 2).

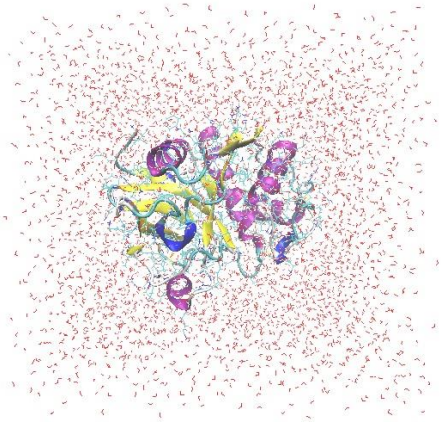

Figure 2. Papain/CLIK148 complex structure in a water box represented in VMD editor

The total system contains 16331 atoms, 4570 papain/CLIK148 complex atoms and 4358 water atoms. The van der Waals interactions were calculated for a cutoff of 12 Å and the electrostatic interactions were calculated using the Particle Mesh Ewald method [16]. A multiple timestepping algorithm [17] used a 2 fs per step.

MD simulations were performed in a NPT ensemble (pressure = 1 atm, temperature = 310 K), in which the pressure was controlled by the hybrid Nosé – Hoover Langevin piston method [18] and the temperature through Langevin dynamics. A free dynamics simulation for the entire system was performed for 200 ps to obtain the equilibrium structure [19].

2.2. Steered Molecular Dynamics (SMD)

Certain conformational changes in biomolecules can be investigated using steered molecular dynamics. In our simulations, we have used the constant velocity pulling method [14]. To develop this method, water molecules were removed from our equilibrated protein structure to obtain a pdb file, which is used to establish the fixed atom (Cα from the first residue: ILE1: CA) and the SMD atom (Cα from the last residue: ASN212: CA).

In this simulation, the SMD atom is attached to a dummy atom through a virtual spring. The virtual spring between the dummy atom and the SMD atom has a spring constant of 7 kcal/mol/$Å^2$, where 1 kcal/mol = 69.479 Å. The pulling was performed at a constant velocity of 0.005 Å/time-step, equivalent to 2.5 Å/ps [14]. SMD simulations for the unfolding process of papain/CLIK148 complex structure were performed during 100 ps.

3. Result and Discussion

3.1. Equilibrium analysis

Our minimization-equilibration simulation for 200 ps with periodic boundary conditions has been generated a trajectory for the entire system [14]. This trajectory is used to calculate the Root Mean Square Deviation (RMSD) of the protein structure during equilibration (Figure 3).

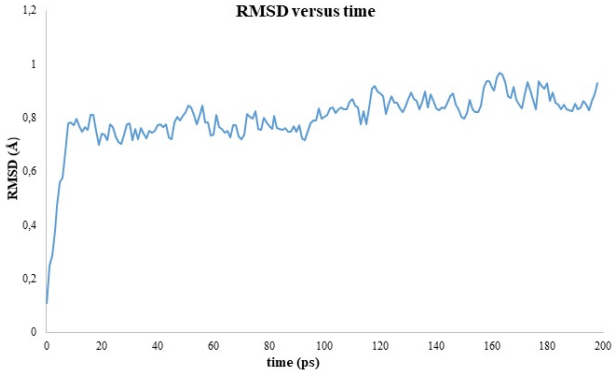

Figure 3. RMSD versus simulation time for papain/CLICK148 complex structure

Figure 3 shows that our protein system exhibits a low energy state and it is conformationally stable. So, the RMSD curve is flatted and our protein system is equilibrated.

Usually, the kinetic energy distribution of the atoms in a system is associated with the Maxwell distribution for a certain temperature [14]. Figure 4 is shown the histogram plot of the energy of papain/CLIK148 complex structure and fitted with a Maxwell-Boltzmann distribution.

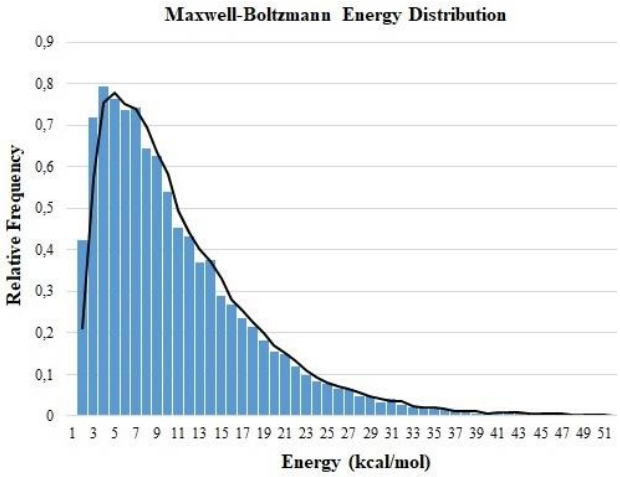

Figure 4. Maxwell-Boltzmann distribution for kinetic energies of papain/CLIK148 complex structure

This plot was fitted using a Maxwell-Boltzmann distribution by the following formula:

$$f(E_k)=\frac{2}{\sqrt{\pi}}\frac{1}{(k_BT)^{\frac{3}{2}}}\sqrt{E_k}\exp(-\frac{E_k}{k_BT}) \quad (1)$$

where, $k_B=0.00198657\ \frac{kcal}{mol\cdot K}$.

It was obtained the value of 310 K for the average temperature, which we have considered in our simulations.

Meanwhile, the fluctuations of kinetic energy are correlated with the ones of temperature. Therefore, we have considered our protein system in a microcanonical ensemble (NVE, i.e., constant number, volume and energy) [14]. The fluctuations of temperature are shown in Figure 5 as a histogram plot of the temperature, which has been fitted with a Gaussian distribution.

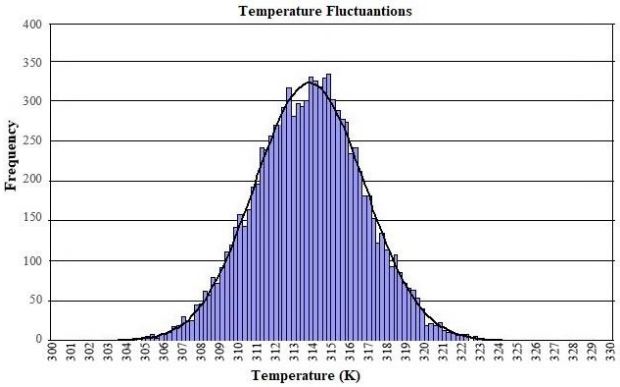

Figure 5. Fluctuations of temperature in a finite sample of papain/CLIK148 complex structure

Thus, the fluctuations of temperature in a finite sample highlight an accurate correlation with the ones of the kinetic energy of the papain/CLIK148 complex structure, which behaves as its thermometer.

3.2. Non-equilibrium analysis

Heat diffusion is one of the most important non-equilibrium properties of proteins. We have simulated the cooling of the papain/CLIK148 complex in the temperature range of 200-338 K. The cooling of the papain/CLIK148 complex in a water box is shown in Figure 6.

It can be noticed that the optimum temperature for papain/CLIK148 complex is 338 K, which corresponds to 65°C. At low temperature (around -

13°C) the protein structure is denatured. These results are according to data from literature [20].

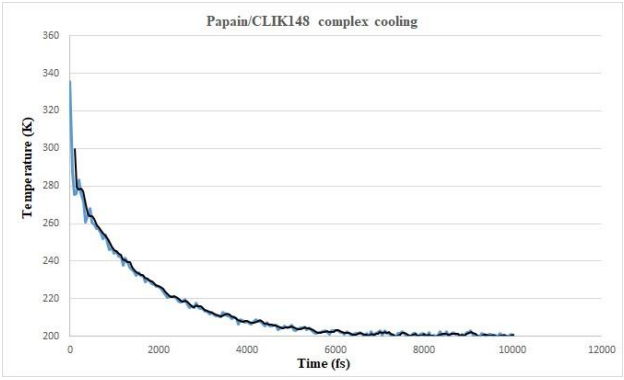

Figure 6. Cooling of papain/CLIK148 complex in a water box. The smooth black line shows a linear fit of the data to the theoretical expression

3.3. Tensile tests

Our constant velocity SMD simulation has generated a trajectory of the unfolding process of papain/CLIK148 complex structure. From this trajectory, we could extract the force applied to the SMD atom. The evolution of this force in time is plotted in Figure 7.

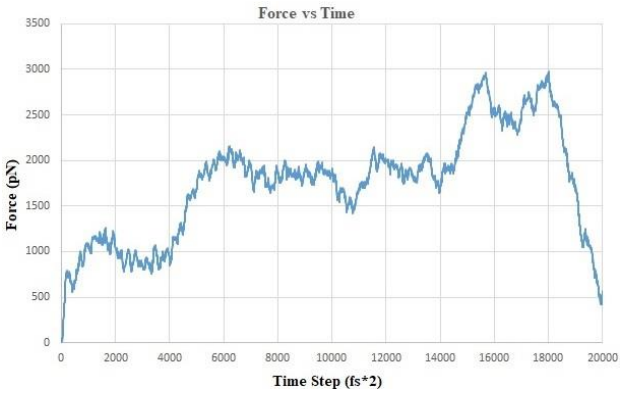

Figure 7. Force versus time for papain/CLIK148 complex structure

By plotting force versus time (Figure 7), we could identify the intermediate states in the unfolding pathway of our protein structure. Three peaks in the force are correlated to the breaking of hydrogen bonds from the mixed β-sheet with seven strands. The low value of force at the end of constant velocity SMD simulation denotes that the protein is not completely unfolded. Thus, it is necessary to run our simulations for a long time until the protein structure is fully unfolded.

4. Conclusion

Plant biostimulants are a new challenge for the agriculture sector. They are substances obtained from natural sources with an important potential on plant growth and development, stress resistance and crop yield. Usually, plant biostimulants are obtained both non-microbial and microbial procedures. Thus, the most used plant biostimulants are protein hydrolysates (PHs) which consist of a mixture of peptides and amino acids. Ones of the most studied are cysteine proteases (Cys residue at the catalytic site) such as chymotrypsin, papain and bromelain, which can be used as biostimulants in horticulture. Lately, various bioinformatics tools (such as semiempirical methods and molecular dynamics simulations) have been used to investigate the structural and conformational changes of the papain.

In this paper, we have developed an atomistic model to study the mechano-chemical properties of papain through molecular dynamics simulations using NAMD 2.13 software. In our molecular dynamics simulations, we have considered the complex structure of papain with CLIK148, which is an experimental model structure to understand the cathepsin L. inhibitors. We have gained new insights into the mechano-chemical properties of this challenging biostimulant used in agriculture. We could identify potential conformational changes of this protein structure in case of various mechanical stresses. Also, we have explored different equilibrium/non-equilibrium properties of the papain/CLIK148 complex, such as energy and temperature distribution and heat diffusion, respectively.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest.

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