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Computational approach to elucidate the thermal stability of *Candida antarctica* lipase B(CALB).

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***Abstract*— *Candida antarctica* Lipase B (CalB) is a significant biocatalyst in a variety of industrial applications due to its exceptional thermostability and catalytic flexibility. Still, a thorough comprehension of the molecular principles behind its remarkable performance at various temperatures is unclear. This work explores the complex interactions among temperature, motion, and functioning in CalB by combining sophisticated computational and experimental methods. Using extended temperature range molecular dynamics simulations, we investigate in detail the impact of thermal stress on the solvent interactions, flexibility, and structure of CalB. Through a detailed examination of structural variations and dynamic characteristics, we hope to unravel the essential movements and interactions that control the thermostability and catalytic activity of CalB at various temperatures. To confirm the simulations and reveal the underlying processes, a comparison study will be conducted using experimental data on stability and activity. This work has great potential to further biocatalysts research and enable the possibility of the creation of innovative, temperature-resistant enzymes for use in the industrial sector by elucidating the complex thermal response of CalB.**

Keywords—Candida antarctica Lipase B(CALB), Molecular dynamics simulations, enzyme.

I. INTRODUCTION

The study of enzyme behavior under various settings has significant significance for improving industrial processes in the dynamic field of biotechnology. Candida antarctica Lipase B (CalB) is a versatile biocatalyst that has attracted a lot of research because of its wide substrate specificity and high catalytic efficiency. However, difficulties related to thermal instability prevent it from reaching its full potential. Using molecular dynamics simulations, this work explores in detail how CalB responds to temperature changes, providing insights into the nuances of its dynamics, structure, and activity.[1]

The study is essential since temperature is a major factor in determining the stability and effectiveness of enzymes. The capacity to control enzyme activity at different temperatures is essential for optimizing industrial processes in biotechnological applications.[2] The stability and catalytic efficacy of CalB are directly impacted by its thermal dynamics, which makes an understanding of them crucial. This study uses molecular dynamics simulations to investigate how temperature affects CalB, which will provide a basis for competent protein engineering techniques to improve thermostability.

Our primary objective is to use molecular dynamics and mutagenesis simulations to study the effects of temperature on the structure and flexibility of CalB. This project aims to achieve these objectives by an examination of the molecular mechanisms behind the structural alterations of CalB in reaction to variations in temperature. Additionally, enzyme assay will demonstrate the relationship between the catalytic activity and the dynamic behavior of CalB, providing important insights into the temperature-dependent functional dynamics required for maximum efficiency which in turn offers new possibilities for efficient and sustainable industrial applications.[3]

II. METHODOLOGY

Molecular dynamics (MD) is a computer simulation that works with biological molecules like proteins and nucleic acids and visualizes their movement in atoms and molecules.[4] These atoms and molecules are used in computer simulations because they may interact with one another throughout time and determine the dynamic evolution of the system. By simulating the changes in biological molecules' structures over time, MD modelling provides us with atomic-level insights into such changes. These data aid in our comprehension of biological processes. [5]

We can learn a great deal about the flexibility and volatility of the proteins and nucleic acids under investigation thanks to these simulations. These methods are used to investigate in detail how biological molecules are organized and behave, as well as how they form complexes and undergo conformational changes in proteins and nucleic acids.[6] In computational biology, MD simulations are frequently used to provide a thorough knowledge of the interactions between proteins and their ligands, address how flexible these interactions are, and shape conformational changes in molecules upon the introduction of certain mutations.[7]

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A. Dataset

A significant step in molecular dynamic simulation is the selection of a suitable target.[8] Through extensive literature survey, the Crystal structure of CALB from Candida antarctica with 1,2 Ethanediol as ligand (Xie, Y et al., 2014) was identified. The structure of the selected molecule (PDB-ID:4K6G) was retrieved from Protein databank (Fig. 1).

B. Preparing the system

Using "pdb2gmx", pdb is now converted to a gromos file (gro). Additionally, pdbgmx generated a topology file (.top). The box type and size that will be utilized in the simulation will be decided at this stage by the "editconf".[9] There are three different kinds of boxes on Gromacs: octahedron, cubic, and triclinic.[10] The protein in the box must be solvated next. Genbox, the programme, will handle it. Depending on the type, Genbox will produce a box described by editconf. Additionally, Genbox decided on the kind of water model to be employed and included the quantity of water molecules needed for solvate proteins. SPC (Simple Point Charge) is the water model that is most frequently utilized.[11]

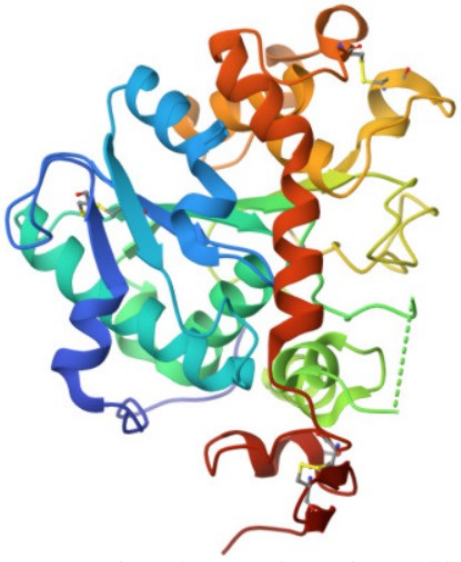

Fig 1. PDB structure of Crystal structure of CALB from Candida antarctica with 1,2 Ethanediol: The ligand (1,2 Ethanediol) is represented by ball-and stick model in contrast to the polymer represented by cartoon structure.

Energy minimization is essential because the introduction of hydrogen bonds or termination in the protein structure may result in atoms being too close, leading to collisions. To address this issue, Gromacs employs an MDP file to set up parameters, specifying the number of steps and cutoff distance. The process involves using grompp to generate an input file and mdrun to execute energy minimization. The duration of energy minimization varies and depends on the CPU used. In the NVT ensemble, the number of particles (N), volume (V), and temperature (T) are kept constant during equilibration. This ensemble is suitable when the system is isolated from its surroundings, and there is no exchange of heat with the environment. During NVT equilibration, the system's kinetic energy is controlled to maintain a constant temperature. Algorithms such as the Nose-Hoover thermostat or the Langevin thermostat are commonly used for this purpose. These algorithms regulate the velocities of particles to achieve the desired temperature while keeping the volume of the system unchanged.[12]

C. Production run

In the NPT ensemble, the number of particles (N), pressure (P), and temperature (T) are kept constant during the equilibration process. This mimics conditions like those found in the real world, where the system is in contact with a constant temperature and pressure reservoir. The equilibration involves adjusting the system volume to reach the desired pressure and temperature. This is typically achieved using algorithms like the Berendsen thermostat and barostat or the Nose-Hoover thermostat and Parrinello-Rahman barostat. These algorithms control temperature and pressure by scaling velocities and adjusting box dimensions.[12] The molecular dynamics simulation follows a similar procedure to energy minimization. Grompp is responsible for preparing the input file for mdrun execution, and the setup parameters are specified in an MDP file. The majority of mdrun options used in molecular dynamics are analogous to those in energy minimization, apart from the "-x" option, which is employed to generate a trajectory file.[13]

III. RESULTS AND DISCUSSIONS

The apo form of the CALB protein was utilized to investigate the dynamics of atoms and analyses structural and conformational changes within the system entities through a 2ns molecular dynamics simulation. Various structural parameters, such as Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), and Radius of Gyration (Rg), were examined to assess the trajectory's stability, flexibility, the affinity of small molecules with the receptor, and the extent of compactness and folding behavior.

A. Potential Energy

The chart illustrates the variation in potential energy of a molecule over time at distinct temperatures. Time, denoted in picoseconds (ps), is depicted along the horizontal axis, while the actual energy, measured in kilojoules per mole (kJ/mol), is presented on the left side. Each of the four colored curves corresponds to a specific temperature, with the highest (330K) positioned at the top and the lowest (300K) at the bottom. Interestingly, all lines originate near zero and progressively ascend over time, indicating an overall increase in the molecule's potential energy regardless of temperature. The particularly noteworthy is that the line representing the highest temperature reaches the peak, indicating that as the molecule's temperature rises, its potential energy also increases. Although the distinctions between the lines are subtle, it is evident that temperature plays a role in determining the magnitude of potential energy the molecule can accumulate. (Fig 2.)

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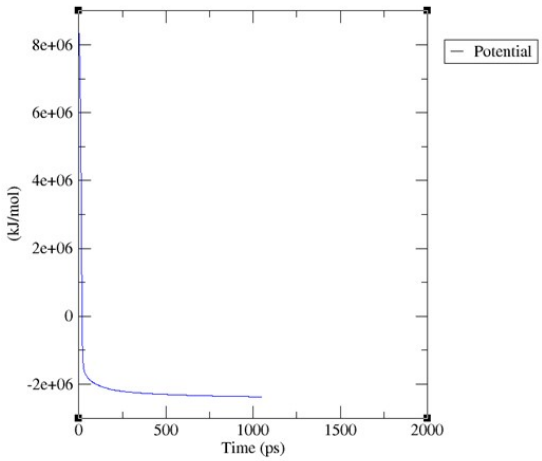

Fig 2. Potential energy of CALB in 300K,310K,320K and 330K

B. *Temperature*

Elevated temperatures (320K and 330K) exhibit significantly increased potential energy in comparison to lower temperatures (300K and 310K). This observation indicates that a temperature rise weakens the intramolecular interactions that contribute to the stabilization of CALB, potentially resulting in reduced stability. Despite the observed trend, the overall disparity in potential energy between different temperatures is relatively modest. This suggests that CALB may retain a certain level of stability even at higher temperatures. The trend, wherein potential energy rises with increasing temperature, implies that higher temperatures might render CALB more susceptible to unfolding or the loss of its native structure.(Fig 3.)

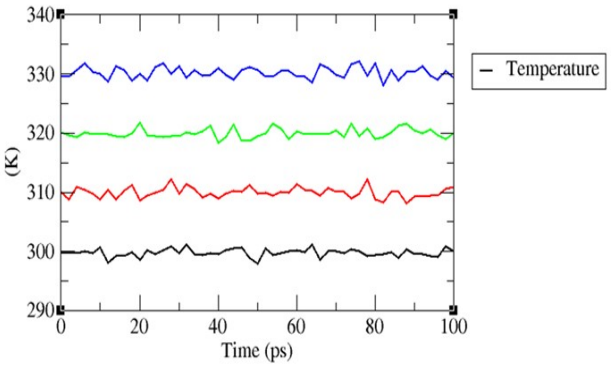

Fig 3. Temperature of CALB at 300K,310K,320K,330K

C. *Root Mean Square Deviation*

Based on the visual representation, it can be inferred that the Root Mean Square Deviation (RMSD) values for the CALB protein backbone at different temperatures are as follows: 0.15 nm(300K),0.17 nm(310K),0.19 nm(320K),0.21 nm(330K).

These values indicate a progressive increase in RMSD, representing the average displacement of the protein backbone atoms from their reference structure, with rising

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mperatures.[14] This implies heightened flexibility and ore significant structural fluctuations in the CALB protein elevated temperatures, aligning with the trend observed in e potential energy curves previously provided. Despite the creasing RMSD values, they remain relatively small ross all temperatures, suggesting that the CALB protein aintains its overall structure even at higher temperatures. he gradual nature of the RMSD increase indicates that the otein does not undergo significant unfolding transitions ithin the simulated temperature range. (Fig 4.)

. *Root Mean Square Fluctuation*

he RMSF values at all four temperatures span a range of 05 nm to 0.21 nm, indicating that protein residues undergo nall to moderate fluctuations throughout the simulations. enerally, higher temperatures correspond to elevated RMSF values, suggesting increased flexibility of the protein and more unrestricted movement of residues as the temperature rises.[15] This observation aligns with the patterns identified in the potential energy and RMSD plots previously presented.

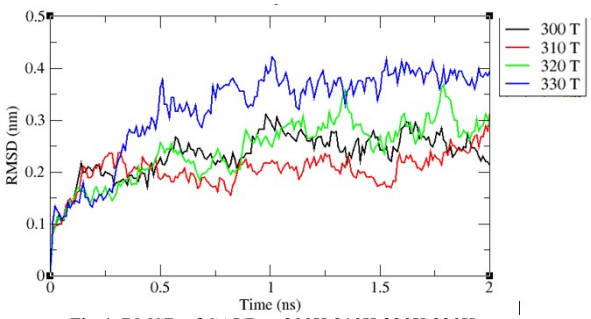

Fig 4. RMSD of CALB at 300K,310K,320K,330K

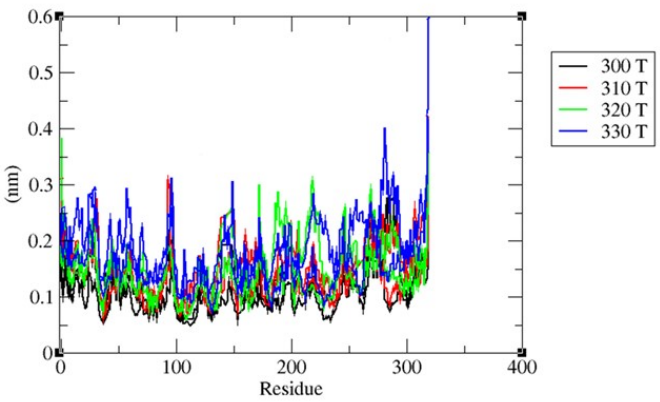

Fig 5. RMSF of CALB at 300K,310K,320K,330K

The fluctuations appear to be confined to specific regions of the protein, as evident from the varying heights of the bars in the graph. Residues exhibiting higher RMSF values demonstrate greater flexibility and dynamism, while those with lower values exhibit more rigidity and stability. (Fig 5.)

E. *Radius of Gyration*

The radius of gyration exhibits a minor increment with rising temperature, expanding from 2.65 nm at 300 K to

2.71 nm at 330 K. This implies a slight expansion of the CALB protein as the temperature rises, indicating a potential relaxation of its overall structure. This observation aligns with the patterns identified in the potential energy, RMSD, and RMSF plots previously presented. Despite the temperature-induced increase in the radius of gyration, the overall change is relatively small, suggesting that the protein sustains its folded state even at higher temperatures.[16] (Fig 6.)

IV. CONCLUSION

CALB, or Candida antarctica lipase B, is a versatile enzyme renowned for its catalytic prowess in various biochemical processes.[17] Derived from the yeast Candida antarctica, CALB belongs to the family of lipases, enzymes that facilitate the hydrolysis of ester bonds in lipids. Its unique structure and functionality have made it a focal point in biocatalysis, particularly in the fields of organic synthesis, pharmaceuticals, and biofuels.[18] The temperature-dependent structural dynamics of CALB through molecular dynamics simulations, spanning temperatures from 300K to 330K. By analyzing potential energy curves, RMSD, and RMSF values, we seek to elucidate the interplay between temperature and protein stability, providing insights into CALB's resilience and flexibility under varying thermal conditions, with implications for biocatalysis and enzyme engineering.

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