Molecular Dynamics Studies on Engineered Industrially Important Proteins

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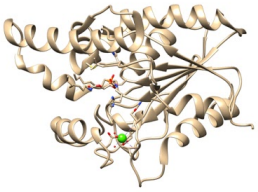
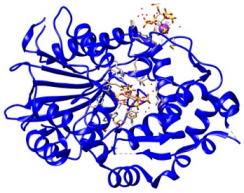
Abstract

Rational designing and engineering of proteins have been a popular endeavor in recent years, especially for proteins of high importance in biotechnology and specialty chemicals. Current researches have been able to produce engineered phytases and lipases with higher activity and specificity than their native forms. These native enzymes have been engineered through directed evolution or rational design. In this work, computational biophysical approach was used to evaluate the structural stability of two enzymes—a phytase from *Hafnia alvei* and lipase from *Pseudomonas aeruginosa*—exposed to high-throughput proline scanning of selected secondary structures. Different systems were set up using the native and mutant forms of the enzymes: phytase in pure water, and lipase in pure water and in pure hexane. Mutations for the phytase were made using Scwrl4, while setting up of the systems, as well as mutations for the lipase, were done using AMBER14. For each system, short-time molecular dynamics (MD) simulations were performed using NAMD2.10. The data obtained will be subjected to different statistical analyses in order to determine the possible consequences of the mutations done. The results of this study will be used to design enzymes with better catalytic properties than the native forms.

Systems of Interest



Pseudomonas aeuroginosa PAO1 lipase (PDB:1EX9)

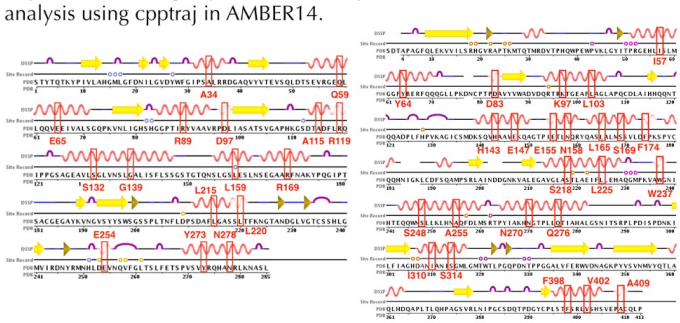


Hafnia alvei phytase (PDB:4ARO)

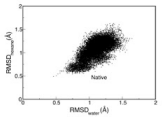
Methods

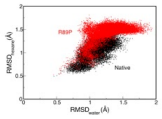
The phytase crystal structure (PDB: 4ARO) [1] was completed using the program MODELLER in Chimera. The site-specific mutations for the phytase were done using the program Scwrl4. For the lipase, mutations were done by modifying the lipase crystal conformation (PDB: 1EX9) [2,3] file. The selection for the secondary structures mutated was based on DSSP Secondary Structure [4].

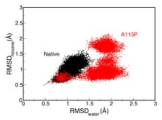
The protein systems were set up using the program xleap in AMBER14 with *ff14SB* as the force field for the peptides, and TIP3P for water. Simulation steps were done in the following sequence: minimization, heating, equilibration, and production using NAMD2.10. Data frames were collected from the production runs at 1 ps intervals (every 500 steps). The resulting trajectories for the production runs were used for data analysis using cpptraj in AMBER14.

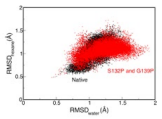


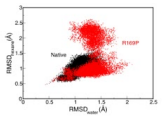
2D-RMSD



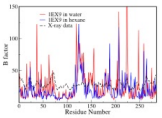


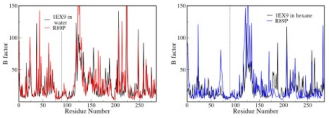


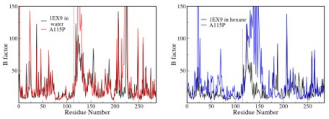


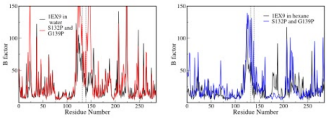


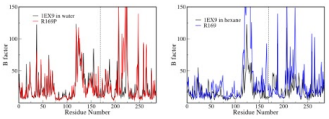
B-factor Analyses











Conclusion

For the *Pseudomonas aeuroginosa* lipase, not all destructive mutations lead to global conformational change. So far, the observed important mutations that alter the overall conformation of the lipase are alpha-3 (R89P), alpha-4 (A115P), alpha-5 (S132P and G139P), and alpha-7 (R169P). Previous studies point out that alpha-5 is the most crucial structure for conformational switching. However, our preliminary results show that other smaller alpha-helical structures are as important as alpha-5.

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