Structural Study of Enzymes in Rigidifying Environments

by

Diana Ivonne Paredes

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Examining Committee:

Jonathan S. Dordick, Thesis Adviser

Christopher Bystroff, Member

Shekhar Garde, Member

Curt Breneman, Member

Rensselaer Polytechnic Institute
Troy, New York

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ABSTRACT

Enzymes find many applications in industry due to their increased efficiency and specificity as compared to most ordinary inorganic catalysts. However, there are technical and economical disadvantages of using free enyzmes in solutions. For example, there is a low recovery rate and a low stability of the enzymes. Therefore, in order to improve the inherent disadvantages of using free enzymes for biocatalytic reactions, new techniques (absorption onto glass, entrapment, cross-linkage, organic solvents, etc.) that immobilize the enzyme have been developed. This immobilized environment has been shown to rigidify the enzyme by restricting its mobility. Consequently, immobilization typically leads to increased enzyme stability, usually at the expense of enzymatic activity.

In order to understand and optimize catalysis in rigidifying environments, we performed two studies; one study focused on cold-adapted enzymes in their natural rigidifying environment, and the other study examined green fluorescent protein (GFP) in an artificial rigidifying environment (GFP attached to agarose beads). Cold-adapted enzymes, known as psychrophiles, are known to be active at cold temperatures due to their increased molecular flexibility. . However, the precise structural adaptations that allow psycrophiles to perform in cold temperatures are not well understood. By studying the structural differences between psychrophiles and their homologous mesophilic counterparts, we were able to conclude that psychrophiles have a more hydrated protein core. We hypothesize that this characteristic contributes to their intrinsic flexibility.

In our second study, LOO7-GFP (leave-one-out, strand 7) was immobilized to be used in biosensing applications. LOO7-GFP can be used as a biosensor if the pocket left by the removal of strand 7 is redesigned to attach to antigen peptides. However, the LOO7-GFP shows high background fluorescence after the strand's removal, which decreases the signal-to-noise ratio after complementation with the peptide. We hypothesized that by perturbing the structure of LOO7-GFP, we could lower the background fluorescence. To that end, we immobilized the protein, since previous studies have shown that immobilization can affect stability. Our observations

corroborated this hypothesis. Therefore, by destabilizing the LOO7-GFP, we were able to decrease the background fluorescence and with this increase the signal-to-noise ratio.

This study helped us to elucidate how enzymes could be optimized to work under rigidifying conditions. Between the two studies, this thesis provides insight about what happens at the molecular level when proteins are exposed to rigidifying environments.