Discrete Changes to Specificity and Catalytic Efficiency in Emergence of Terpene Cyclization

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Abstract: The proliferation and rapid refinement of terpenes was crucial for adaptations and survival in plants. Mutations in terpene synthases lead to profound changes in product specificity and reaction rates. We have developed a method to quantitatively characterize Michaelis-Menten enzyme kinetics from high-throughput evolutionary assays. More specifically, we discuss Gibbs energies and the corresponding mutational pathways of a terpene synthase enzyme as it develops the ability to produce a cyclic product (a-bisabolol) starting from an enzyme which is fine-tuned to make a linear product (E-b-farnesene).

Keywords: Mutational pathways, Enzyme kinetics

I. INTRODUCTION

Terpenes are important chemical compounds found in many plants. They have also found multiple industrial and medical uses. For example, artemisinin, a compound produced by A. annua, is the basis for the state-of-the-art anti-malarial drug. The production of terpenes is catalyzed by the class of enzymes called terpene synthases. Terpenes are found to be either linear or cyclic. The reaction rate and the types of product are controlled by the molecular architecture of the substrate-binding interface. We address the question of emergence of terpene cyclization, focusing on mutational pathways as well as enzyme energetics and kinetics.

II. ENZYME KINETICS

In order to determine how product specificity and reaction rates depend on an enzyme sequence, it is imperative to understand enzyme kinetics. [1] According to the standard Michaelis-Menten model, the reaction rate of a product is a function of the difference between two corresponding Gibbs energies: G3 and G4. G3 is product-independent and determines the overall reaction rate, whereas G4 is product-specific.

III. EXPERIMENTAL AND COMPUTATIONAL APPROACH TO STUDYING ENZYME EVOLUTION

We have developed a method which allows us to predict G3 and G4 from the total reaction rate and mass-spec data. The data is obtained using the SCOPE method for controlled protein engineering [2-4]. Multiple rounds of mutation and selection are employed to map out the ensemble of mutational pathways and identify sequence variants with novel enzymatic functions. Functional assays, carried out for more than a hundred sequence variants, involve enzyme characterization by mass-spectroscopy and kinetic measurements. The computational approach involves single-amino acid contributions as well as pairwise couplings between residues. A similar model, inspired by widely-used spin-glass models in statistical physics, has been previously used to study protein-protein interactions [5]. Our predictions yield G3 and G4 predictions for every enzyme characterized in our evolutionary assay. Thus we are able to map out evolution of enzyme energetics corresponding to the emergence of terpene cyclization in a key family of plant enzymes.

IV. CONCLUSION

Our method is useful for studying how evolution of enzyme energetics and kinetics drives emergence of novel catalytic functions in terpene synthases.

REFERENCES

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