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Designed out-of-active-site mutations in human oxidosqualene cyclase modulate the activation entropy and enthalpy of the cyclization reaction

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PURPOSE OF THE ABSTRACT

Abstract
Cholesterol and steroids in general, are essential in all complex life forms. Cholesterol precursors are synthesized from (S)-2,3-oxidosqualene via lanosterol with the aid of the catalyst human oxidosqualene cyclase (hOSC). Due to the essential role of cholesterol in health and disease and large implication an imbalance can have, the enzymes involved in the synthesis of cholesterol have gained increased attention. To understand the mechanism behind the catalysis is essential to understanding the processes and help in understanding the ins and outs of imbalanced cholesterol.
The enzyme involved in synthesizing lanosterol from oxidosqualene, has been well studied and the reaction mechanism has been resolved via QM/MM. However, in these types of enzymes the catalysis and mechanism is depending on two major energetic driving forces, entropy and enthalpy. In this enzyme the reaction is mainly driven by entropy. We hypothesized that the entropy of the reaction is provided by the water molecules present in the enzyme and that the nature of the reaction can be changed. We intended to change the driving force of the reaction to enthalpy via water tunnel analysis, MD simulations and site directed mutagenesis. We aimed to block of tunnels found within the protein with bulky substrates to alter the accessibility of water in the active site.
MD simulations uncovered that in some variants the tunnels were indeed closed and a reduced number of water molecules was around the active site. The reaction kinetics were tested using Eyring transition state theory. With this technique, we can extract the entropic and enthalpic contribution in the catalysis of the enzyme. Indeed what we see is that the changed water contribution in the active site and via these tunnels have an immense impact on the entropy and enthalpy of the reaction. We observed a complete change of entropy of as much as 49 kcal/mol at 300K. The enthalpy changed with similarly high values of 37.5 kcal/mol.
Additionally we tested computational methods to predict variants based on sequence and on provided tunnel blocking mutations. Some of the variants designed had an increased activity of 5-6x. A different subset of variants had an altered temperature dependance, where catalysis within the enzyme went faster at lower temperatures.
We were able to engineer enzymes to have a completely different energetic profile for catalysis, increasing our understanding of these types of enzymes and enabling a novel way to engineer enzymes for increased activity at high and low temperatures.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

BIBLIOGRAPHY