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COMPUTATIONAL INSIGHTS INTO THE MUTATIONS OF AN OMEGA-TRANSAMINASE USING EVOLUTIONARY FOOTPRINTS

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PURPOSE OF THE ABSTRACT

Transaminases are attractive enzymes to produce enantiopure amines. In the last decade, they are increasingly used to synthesize optically pure chiral amines, which are important intermediates in the synthesis of pharmaceuticals and other fine chemicals.[1] However, the limited activity of these enzymes often causes a bottleneck of their application in biocatalysis. For this reason, the design of new enzymes with improved activity and also operability under industrial process conditions is a significant issue. [2] Although a variety of studies focus on the development of new variants by mutations in the active site of the enzymes, it remains inadequately understood how beneficial the mutations far from the active site confer improved catalytic properties. Getting an insight into this issue would aid the design of new enzymes with higher catalytic activities. Moreover, for natural evolution, it is also shrouded in mystery how mutations far from the active site affect the active sites' functions and also stability. Thus, the current challenge is to figure out how nature alters the function and biophysical properties of the enzymes by amino acid substitutions in both allosteric sites and active sites during evolution.

In this study, the objective is to develop an understanding of the mutations that occurred during the natural evolution of a member of the omega-transaminases family, using computational tools and developing a new design conception. With this study, it is also possible to understand the role of cooperativity between allosteric sites and active sites of the enzymes in functionality. To achieve the stated purposes, a modern-day 4-aminobutyrate transaminase, and its 4 ancestral variants having improved catalytic activity and higher promiscuity in substrate selectivity [3] were chosen to study. All mutations that occurred during their evolution were outside the active sites of the enzymes and active sites were totally conserved. Functional collective motions within the variants were evaluated by using computational methods, such as Gaussian Network Model (GNM) and Anisotropic Network Model (ANM). Effects of the mutations that occurred at the dynamic domain interfaces were put under the scope so that mutations could be classified and the important ones for the catalytic activity in addition to substrate selectivity could be identified. Molecular dynamic simulations were performed to develop an understanding of the interactions within and nearby the active sites, as well as the changes in the global behavior of the variants. With this work, the computational results obtained from the stated methods will be presented and discussed.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS
evolution | transaminase | mutation | enzyme activity

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