

N°1323 / PC

TOPIC(s) : Enzyme discovery and engineering

Mechanistic investigation of the kinetic resolution of α -methyl-substituted phenylacetaldehyde by norcoclaurine synthase

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PURPOSE OF THE ABSTRACT

The norcoclaurine synthase from *Thalictrum flavum* (TfNCS) can stereoselectively catalyze the Pictet-Spengler reaction between dopamine and 4-hydroxyphenylacetaldehyde to give (S)-norcoclaurine which acts as an important intermediate in benzyloquinoline alkaloid biosynthesis [1,2]. Recently, the kinetic resolution of α -methyl-substituted phenylacetaldehyde was achieved by TfNCS with high stereoselectivity and yields the (1S, 2R) chiral product [3]. However, the reaction mechanisms and the origins of enantiopreference of TfNCS towards α -methyl-substituted phenylacetaldehyde are still unclear. Herein, a cluster model of the enzyme was designed on the basis of the crystal structure and quantum chemical calculations were then performed to obtain the detailed reaction mechanism and energy profiles. Our calculations reveal that the reaction of dopamine with α -methyl-substituted phenylacetaldehyde by TfNCS follows a similar mechanism as the natural substrate, in which the deprotonation of C-H of the cyclized intermediate is the rate-limiting step for both R- and S-pathways, and the corresponding energy barriers are 20.1 and 21.6 kcal/mol, respectively. The results are thus able to reproduce the experimental results. Importantly, the calculations could also rationalize the observed enantioselectivity of TfNCS towards the non-natural substrate. By analyzing the geometries of the intermediates and transition states, the M97 and L72 residues are indicated as the important residues in controlling the selectivity of TfNCS on α -methyl-substitute phenylacetaldehyde. The detailed information about the mechanism and the enantioselectivity is helpful to rationally design variants of TfNCS with improved reactivity and selectivity properties for a wider scope of substrates.

FIGURES

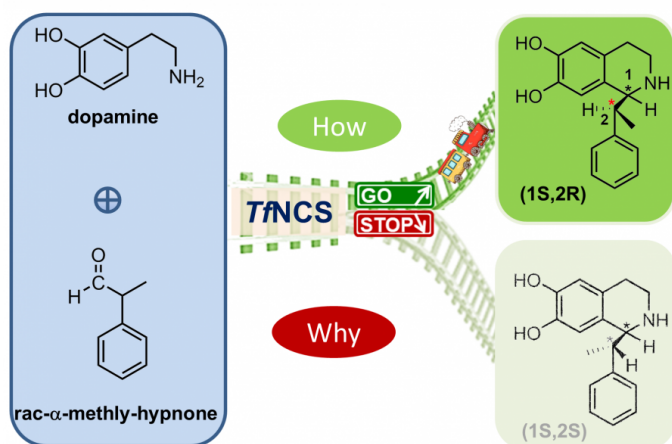


FIGURE 1

Figure 1

The kinetic resolution reaction catalyzed by TfNCS.

FIGURE 2

KEYWORDS

norcoclaurine synthase | reaction mechanism | enantioselectivity | quantum chemical calculation

BIBLIOGRAPHY

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