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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in School of Life Science and Technology 系 コース
学生氏名： Student's Name AFIFA AYU KOESOEMA
申請学位（専攻分野）： Academic Degree Requested 博士 Doctor of (Engineering)
指導教員（主）： Academic Supervisor(main) Associate Professor Tomoko Matsuda
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

In the past decades, biocatalysis has been utilized for organic synthesis due to its environmentally-friendly and highly enantioselective nature. Alcohol dehydrogenases (ADHs) catalyze the reversible reduction of a carbonyl group to the corresponding alcohol and can be utilized as an alternative of the conventional catalysts. ADHs have broad substrate specificity and high enantioselectivity. Moreover, by using ADHs possessing opposite enantiopreference, it is possible to access both (*S*)- and (*R*)-chiral alcohols beneficial for various drug intermediates. In this study, the potential of organic synthesis catalyzed by a novel and robust ADH, *Geotrichum candidum* acetophenone reductase (*Gc*APRD), is investigated. Rational design was performed to alter the substrate specificity and the enantioselectivity of the *Gc*APRD wild type. At last, the mechanism underlying the observed enantioselectivities of *Gc*APRD wild type and mutants were investigated by using X-ray structure determination and docking simulations.

*Gc*APRD wild type reduced "easy-to-resolve" ketones such as halogenated acetophenone, and tetralone derivatives with high yield and excellent (*S*)-enantioselectivity. It also reduced extremely "difficult-to-resolve" ketone owning only one carbon difference among substituents to the carbonyl carbon, such as 3-hexanone (ethyl propyl ketone) with >99% ee (*S*). This reduction enantioselectivity is the highest to be reported among chemical catalysts and biocatalysts, to the best of my knowledge. Despite the advantages shown by the *Gc*APRD wild type, it has a limitation in reducing "bulky-bulky" ketones, owning both bulky substituents to the carbonyl carbon, and it cannot produce the corresponding (*R*)-alcohols.

Rational design was then performed on residue Trp288, which limits the small binding pocket size of *Gc*APRD. The substrate specificities of *Gc*APRD Trp288 mutants was expanded toward bulky-bulky ketone, unable to be reduced by the wild type. Their enantioselectivities were found to be highly dependent on the ketones' substituent. For example, in the reduction of aliphatic ketones, when one of the substituents to the carbonyl carbon was elongated from propyl to butyl and pentyl, enantioselectivity inversion from (*S*) to (*R*) was observed. Further elongation of one of the substituents to hexyl or longer inverted the enantioselectivity back to (*S*), which was first to be reported, to the best of my knowledge. High (*R*)-enantioselectivity was also observed for the reduction of ketone by Trp288 mutants. For example, the reduction of 3-octanone by Trp288Val mutant proceeded with 97%

ee (*R*), in contrast with the reduction by the wild type which proceeded with >99% *ee* (*S*). The reduction of 4-octanone (propyl butyl ketone), a substrate which is both “difficult-to-resolve” and “bulky-bulky, by Trp288Val mutant proceeded with 87% *ee* (*R*), which was the first to be reported to the best of my knowledge.

X-ray crystallography to determine the structure of the *Gc*APRD wild type was then performed to elucidate the observed enantioselectivity phenomenon shown by the *Gc*APRD wild type and Trp288 mutants. *Gc*APRD wild type possessed similar folds with other medium-chain dehydrogenases. However, both the outer loops and active site of the *Gc*APRD wild type were found to be different from its closest homolog, chain C of (*R*)-specific carbonyl reductase from *Candida parapsilopsis* (RCR), indicating its novelty. Following the structure determination, docking simulations were performed to understand the mechanism underlying the enantioselective reduction of “difficult-to-resolve” ketones such as 3-hexanone by *Gc*APRD wild type. Docking simulations revealed an important stabilizing C-H···π interaction between C_β of ethyl substituent in 3-hexanone and Trp288.

Next, docking simulations were also performed to understand the enantioselectivity inversion observed in Trp288 mutants, such as in the reduction of 4-octanone. For aliphatic ketones reduction, docking simulations suggested that only one crucial interaction between Gly94 and butyl or pentyl substituent of ketone was found to be necessary for the enantioselectivity inversion from (*S*) to (*R*). For example, the pro-*R* binding pose 4-octanone in the Trp288Val model structure showed a stabilizing van der Waals interaction between the C_α of Gly94 and the butyl substituent of 4-octanone. The example underlined the importance of the interaction of the substrate substituent to determine the enantiopreference in *Gc*APRD Trp288 mutants-catalyzed reduction of ketones.

In summary, the *Gc*APRD wild type is a versatile catalyst to reduce both “easy-to-resolve” and“difficult-to-resolve”ketones with excellent (*S*)-enantioselectivities. The limitation of the *Gc*APRD wild type was overcome by rational design on residue Trp288, which limits the size of the small binding pocket of the *Gc*APRD. Trp288 mutants were able to reduce bulky-bulky ketones with high (*R*)-enantioselectivities. Structural study and docking simulations revealed the mechanism underlying the observed enantioselectivity in both wild type and Trp288 mutants. In general, the interaction between the ketone substituent with the substrate-binding pocket is crucial to determine the reduction enantioselectivity of *Gc*APRD wild type and mutants. Knowledge of such precise enantioselectivity mechanisms found in *Gc*APRD can contribute to the mechanism elucidation of ADHs. In the future, I believe that it can also be applied to tailor other enzymes with desired substrate specificity and enantioselectivity.