論文 / 著書情報
Article / Book Information

題目(和文)	アルコール脱水素酵素の基質特異性および立体選択性の制御および機構解明
Title(English)	Control and Mechanism Elucidation of Alcohol Dehydrogenase ' s Substrate Specificity and Enantioselectivity
著者(和文)	クスマアフィファ アユ
Author(English)	Afifa Ayu Koesoema
出典(和文)	学位:博士(工学), 学位授与機関:東京工業大学, 報告番号:甲第11483号, 授与年月日:2020年3月26日, 学位の種別:課程博士, 審査員:松田 知子,中村 聡,福居 俊昭,和地 正明,上野 隆史
Citation(English)	Degree:Doctor (Engineering), Conferring organization: Tokyo Institute of Technology, Report number:甲第11483号, Conferred date:2020/3/26, Degree Type:Course doctor, Examiner:,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	要約
Type(English)	Outline

Control and Mechanism Elucidation of Alcohol Dehydrogenase's Substrate Specificity and Enantioselectivity

Afifa Ayu KOESOEMA

Academic Supervisor: Associate Professor Tomoko MATSUDA

Introduction

Alcohol dehydrogenase (ADH) is beneficial for the synthesis of chiral alcohols for various intermediates. In this study, a novel *Geotrichum candidum* acetophenone reductase (*Gc*APRD) is utilized for enantioselective reduction. The substrate specificity and enantioselectivity are further controlled through rational design. Structural analysis and docking simulations were performed to elucidate the mechanism of *Gc*APRD.

Results and Discussion

1. Enantioselective reduction by *Gc*APRD wild type

Most ADHs, including *Gc*APRD wild type, can reduce "easy-to-resolve" ketones with significant size differences between substituents to the carbonyl carbon. On the other hand, the reduction of "difficult-to-resolve" ketones with similar substituents to the carbonyl carbon is challenging. *Gc*APRD wild type reduced "difficult-to-resolve" ketones with excellent enantioselectivities. For example, it reduced 3-hexanone (ethyl propyl ketone) with >99% *ee* (*S*), the highest reported among enantioselective reduction by non-enzymatic or enzymatic catalysis, to my knowledge.

2. Engineering of *Gc*APRD by rational design

Despite the advantages of *Gc*APRD wild type, it could not reduce "bulky-bulky" ketones with both bulky substituents to the carbonyl carbon, and could not produce (*R*)-alcohol, although both (*S*)-and (*R*)-alcohols are needed as intermediates. *Gc*APRD Trp288 mutants with an enlarged small binding pocket were created, expanding of the substrate specificity towards bulky-bulky ketones. The reduction's enantioselectivity was dependent on the substrate's substituent. In the reduction of an ethyl ketone series, enantioselectivity inverted from (*S*) to (*R*) when one of the substituents to the carbonyl carbon was elongated from propyl butyl or pentyl. The enantioselectivity inverted back to (*S*), when the substituent was elongated to hexyl, which was the first to be reported, to my knowledge (Fig. **1**). *Gc*APRD Trp288Val mutant also reduced 4-octanone (propyl butyl ketone) with the highest enantioselectivity ever reported, 87% *ee* (*R*).

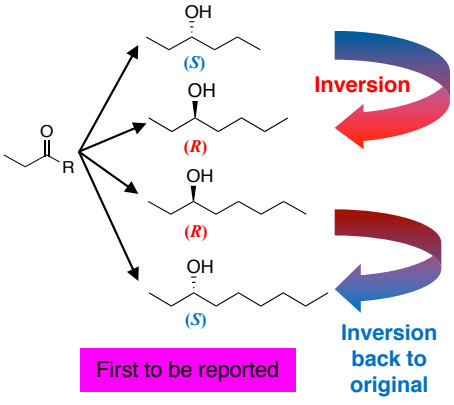

Fig. 1 Enantioselectivity inversion to (*R*) and inversion back to (*S*) in ethyl ketone reduction by *Gc*APRD Trp288 mutants

3. Mechanism elucidation of *Gc*APRD by structural analysis and docking

The crystal structure of *Gc*APRD wild type was solved prior to docking simulations to understand the highly enantioselective reduction of 3-hexanone. Docking simulations indicated that only the pro-*S* binding pose of 3-hexanone was productive to produce the corresponding (*S*)-alcohol, and a C-H···π interaction formed by residue Trp288 was important to stabilize this pro-*S* binding pose (Fig. **2a**). Next, docking simulations were performed to understand the enantioselectivity inversion of *Gc*APRD Trp288 mutants. The pro-*R* binding pose of 4-octanone in the Trp288Val model structure was productive to produce the corresponding (*R*)-alcohol. A van der Waals interaction formed by the C_α of Gly94 and the butyl substituent stabilized the pro-*R* binding pose (Fig. **2b**). Gly94 was located in a loop previously covered by Trp288 in the wild type.

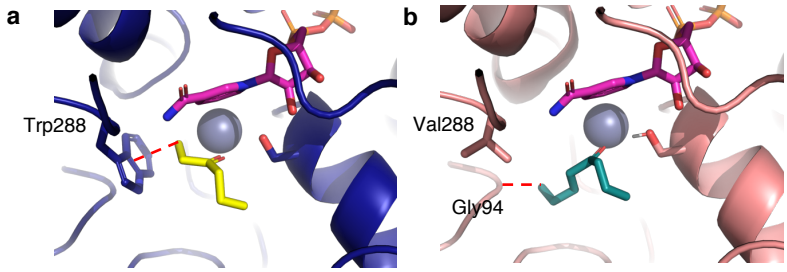

Fig. 2 Binding poses of **a** 3-hexanone in the *Gc*APRD wild type crystal structure and **b** 4-octanone in the *Gc*APRD Trp288Val model structure.

Conclusions

*Gc*APRD wild type reduced "easy-to-resolve" and "difficult-to-resolve" ketones with excellent (*S*)-enantioselectivities. *Gc*APRD Trp288 mutants reduced bulky-bulky ketones with high (*R*)-enantioselectivities. Structural analysis and docking simulations indicated important interactions to explain the observed excellent enantioselectivity and enantioselectivity inversion. This precise enantioselectivity control mechanism is valuable to tailor other enzymes in the future.

Publications

Koesoema AA, Sugiyama Y, Xu Z, Standley DM, Senda M, Senda T, Matsuda T. (2019) Structural basis for a highly (*S*)-enantioselective reductase towards aliphatic ketones with only one carbon difference between side chain. Appl Microbiol Biotechnol, 103:9543-9553. doi:10.1007/s00253-019-10093-w.

Koesoema AA, Sugiyama Y, T.Sriwong K, Xu Z, Verina S, Standley DM, Senda M, Senda T, Matsuda T. (2019) Reversible control of enantioselectivity by length of ketone substituent in biocatalytic reduction. Appl Microbiol Biotechnol, 103:9525-9541. doi: 10.1007/s00253-019-10206-5.

Koesoema AA, Standley DM, T.sriwong K, Tamura M, Matsuda T (2020) Access to both enantiomers of substituted 2-tetralol analogs by a highly enantioselective reductase. Tetrahedron Letters. doi: 10.1016/j.tetlet.2020.151682.

Koesoema AA, Standley DM, Senda T, Matsuda T (2020) Impact and relevance of alcohol dehydrogenase enantioselectivities on biotechnological applications. Applied Microbiology and Biotechnology. doi: 10.1007/s00253-020-10440-2.