Stereoinversion of *R*-Configured Secondary Alcohols Using a Single Enzymatic Approach

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Abstract

Here, we achieved a single-enzymatic stereoinversion of *R*-configured alcohols to their corresponding *S* enantiomers in high percent conversion and high enantioselectivity. A single mutant of *Thermoanaerobacter pseudoethanolicus* secondary alcohol dehydrogenase (TeSADH) oxidized *R* alcohols to their corresponding ketones under conditions that allow selectivity mistakes and then reduced them under stereoselective conditions to generate the corresponding *S*-configured alcohols. The success of this stereoinversion relies on controlling the stereoselectivity of the oxidation and the reduction reactions by varying the amounts of acetone and 2-propanol, respectively. Furthermore, the deployment of the TeSADH mutants W110A and W110G led to stereoinversion of enantiopure *R* alcohols to their *S* enantiomers in medium to high enantiomeric excess (up to >99% *ee*). The disclosed approach of single enzymatic stereoinversion presents a biocatalytic alternative to Mitsunobu inversion reaction.

Introduction

Majority of therapeutic drugs and natural products are active in one enantiomeric form.^[1] The biological targets (receptors, enzymes, transporters) display handedness and therefore drugs must be stereoselective in their interactions. Hence, a plethora of synthetic routes to optically active compounds has been under intensive development. Optically active secondary alcohols are very important synthons in pharmaceutical, agrochemical, and food industries. Among the most important methods in production of enantiomerically pure secondary alcohols are kinetic resolution (KR)^[2] and deracemization.^[3] Deracemization approaches, which include dynamic kinetic resolution, enantioconvergence, cyclic deracemization, and deracemization via stereoinversion, are not easy to design because they require at least two compatible processes to operate in one pot. Moreover, most of the enzymatic deracemization reactions require at least two enzymes with opposite stereopreferences^[4] or whole cells.^[5] Likewise, KR suffers from the limitations of 50% theoretical yield and the requirement of separation of the product from the remaining substrate. It is critical therefore to develop a sustainable and environmentally benign transformation that would invert the configuration of the undesired enantiopure alcohol to the opposite and desired enantiomer. To date, Mitsunobu reaction is the most popular stereoinversion method to generate enantiopure alcohols,^[6] which from the time of its inception has been applied in myriad syntheses.^[7] Despite its broad substrate tolerance and mild reaction conditions, the arduous purification from the generated stoichiometric by-products, phosphine oxide and hydrazine, and the poor atom economy limited the utility of Mitsunobu reaction in industrial applications. As such, quest for alternative approaches to realize this important transformation is continued.^[8]

A concurrent stereoinversion of (*S*)-1-phenylethanol to its *R* counterpart has been accomplished by employing resting cells of *Escherichia coli* that contain alcohol dehydrogenase (ADH) from *Rhodococcus ruber* DSM 44541 (known as ADH-A), *Lactobacillus brevis* ADH and glucose dehydrogenase.^[9] Recently, we reported a deracemization method for secondary alcohols using a single mutant of *Thermoanaerobacter pseudoethanolicus* secondary ADH (TeSADH, EC 1.1.1.2), a nicotinamide-adenine dinucleotide phosphate (NADP⁺)-dependent ADH.^[10] The W110A and W110G mutants of TeSADH exhibited a unique stereoselectivity that was tunable to achieve low or high stereoselective oxidation and reduction by simply varying the amounts of acetone and 2-propanol cosubstrates, respectively. W110 TeSADH mutants follow the Prelog's rule^[11] and are reported in the (*S*)-selective reduction of phenyl-ring-containing ketones.^[12] On the contrary, W110 TeSADH mutants are reported in racemization of *R*- and *S*-configured enantiopure secondary alcohols under conditions that induce selectivity mistakes.^[13]

The ability of W110 TeSADH mutants to recognize the *anti*-Prelog alcohol (i.e., *R* alcohols) via selectivity mistakes prompted us to use them to conduct stereoinversion of *R* alcohols. The stereoinversion, reported herein, relies on oxidation of *R* alcohols to their corresponding prochiral ketones followed by (*S*)-selective reduction of the ketone intermediate, all done using the same enzyme mutant in one pot and without the need to isolate the ketone (Scheme 1).

Scheme 1. Stereoinversion of *R* alcohols by non-selective oxidation followed by *S*-selective reduction.

Results and Discussion

To demonstrate the ability of W110 TeSADH mutants to invert the stereochemistry of *R* alcohols, we studied the extent of W110G TeSADH-catalyzed oxidation of (*R*)-4-phenyl-2-butanol [(*R*)-**1a**], the undesired enantiomer for W110G TeSADH, and the corresponding (*S*)-**1a** at various time intervals (Figure 1). We then extended the same study towards (*R*)-**1a**, using W110A TeSADH or W110V TeSADH mutants. These reactions were performed in tris(hydroxymethyl)aminomethane-HCl (Tris-HCl) buffer solutions containing NADP⁺ and acetone (3% v/v), the latter serves as a cosubstrate as well as a cosolvent. Despite the relatively slower oxidation of the (*R*)-**1a** compared to (*S*)-**1a**, W110G TeSADH showed an excellent performance in an *anti*-Prelog mode, which was evident by the comparable oxidation yields obtained after 12 hours [88% of (*R*)-**1a** versus 90% of (*S*)-**1a**]. These results also revealed that W110G TeSADH and W110A TeSADH were able to deplete the undesired (*R*)-**1a**, contrary to the highly stereoselective W110V TeSADH that exhibited high stereoselectivity for the *S*-enantiomer. In comparison to W110G TeSADH, the lower performance of W110A TeSADH in the oxidation of (*R*)-**1a** can be explained by its relatively higher catalytic efficiency. These results are consistent with those previously reported for the enantiospecific oxidation of 1-phenyl-2-propanol wherein W110G, W110A, and W110V TeSADH mutants displayed enantiomeric ratio [*E* values, $(k_{\text{cat}}/K_{\text{m}})_{\text{S}}/(k_{\text{cat}}/K_{\text{m}})_{\text{R}}$] of 9.0, 17.4, and 134.5, respectively.^[14] However, the currently reported results showed that even a highly selective mutant like W110V TeSADH can encounter selectivity mistakes (Figure 1), but significantly less than those in W110A and W110G TeSADH mutants.

Figure 1. Comparison of oxidation of enantiopure **1a** at different time intervals using W110G, W110A and W110V TeSADHs.

It is worth mentioning that, under the oxidation reaction conditions for (*R*)-**1a**, the *ee* of the unreacted alcohol was <99%. This indicated that the reduction inevitably generated the (*S*)-alcohol from the intermediate ketone, which practically prevented the completion of the oxidation reaction (Figure S2 of Supporting Information).

We extended the oxidation studies to other selected substrates by W110A, W110G and W110V TeSADH mutants, using 3% (v/v) acetone as the optimum cosubstrate concentration as previously reported.^[10] The poor performance of W110V mutant in the oxidation of (*R*)-1-phenyl-2-propanol [(*R*)-**1b**] (18% conversion) and (*R*)-**1a** (12% conversion) (Table 1, entries 3 and 7), as compared to W110A and W110G mutants (>87% conversion), clearly manifested the essential role of the mutant's active site in controlling the stereoselectivity of the oxidation reactions, and thus allowing selectivity mistakes to take place. The poor oxidation by W110V TeSADH is attributed to the reduced selectivity mistakes due to the relatively smaller size of the larger binding pocket in the active site in comparison to those in the case of W110A and W110G TeSADH mutants. The undetectable oxidation of (*R*)-4-phenyl-3-buten-2-ol [(*R*)-**1c**] by all mutants was

linked to the reduced selectivity mistakes, which is caused by its fixed alignment in the active site due to the double bond that inhibits its inability to rotate. This is in line with the previously reported results for W110A TeSADH-catalyzed KR of (*rac*)-**1c** that demonstrated a 50% conversion with >99% *ee*, an indication that (*R*)-**1c** was not detected by W110A TeSADH (i.e., no selectivity mistakes).^[12c] Moreover, W110G TeSADH led to the complete oxidation of (*R*)-4-(4'-hydroxyphenyl)-2-butanol [(*R*)-**1d**] whereas W110A TeSADH displayed a relatively poor performance (Table 1). Likewise, excellent conversions (>99%) were obtained in the oxidation of (*R*)-4-(4'-methoxyphenyl)-2-butanol [(*R*)-**1e**] using both W110A and W110G TeSADH mutants (Table 1).

Table 1. Activity and stereospecificity of W110A, W110G, and W110V TeSADH-catalyzed oxidation of phenyl-ring-containing *R*-configured alcohols.^[a]

Entry	Substrate	R	X	na <i>ee</i> (%) ^[c]	nb (%)
1	(R)- 1a	C ₆ H ₅ CH ₂ CH ₂	W110G	20(<i>R</i>)	95
2			W110A	<i>rac</i>	96
3			W110V	91(<i>R</i>)	12 ^[b]
4	(R)- 1b	C ₆ H ₅ CH ₂	W110G	3(<i>R</i>)	71 ^[b]
5			W110G	15(<i>R</i>)	91
6			W110A	4(<i>R</i>)	87
7	(R)- 1c	C ₆ H ₅ CH=CH	W110V	>99(<i>R</i>)	18
8			W110G	87(<i>R</i>)	<0.5
9			W110A	87(<i>R</i>)	<0.5
10	(R)- 1d	<i>p</i> -HO-C ₆ H ₄ CH ₂ CH ₂	W110V	87(<i>R</i>)	<0.5
11			W110G	-	>99
12			W110A	>99(<i>R</i>)	82
13	(R)- 1e	<i>p</i> -MeO-C ₆ H ₄ CH ₂ CH ₂	W110G	-	>99 ^b
14			W110A	-	>99 ^b

[a] Unless stated, oxidation reactions were performed using 0.03 mmol (*R*)-**1a-e**, 0.4 mg TeSADH mutant, and 1.0 mg NADP⁺ in 1.0 mL Tris-HCl buffer solution (50 mM, pH 8.0) containing acetone (3%, v/v), at 50 °C with shaking

at 180 rpm for 36 h. [b] TeSADH mutant (0.2 mg) and 24 h reaction time. [c] The *ee* of the corresponding acetate ester derivative of the unreacted alcohol was determined by a GC using a chiral stationary phase.

The ability of W110A and W110G TeSADH mutants to oxidize *R*-configured alcohols enticed us to envisage the scheme of a single enzymatic stereoinversion of *R*-configured alcohols, which would involve oxidation of *R* alcohol that is followed by the enantioselective reduction of the ketone intermediate to the corresponding (*S*)-configured alcohol. To optimize this stereoinversion, the oxidation was conducted under the conditions described in Table 1 until no further accumulation of ketone was detected (GC analysis). The equilibrium was then switched to the reduction pathway by adding 2-propanol (30%, v/v), an optimum concentration for (*S*)-selective reduction with high yield.^[10] Stereoinversion of enantiopure (*R*)-**1a** resulted in 80% *ee* and 86% *ee* of (*S*)-**1a** by using W110G and W110A TeSADH mutants, respectively (Table 2). Under identical conditions, stereoinversion of (*R*)-**1b** by the same mutants yielded (*S*)-**1b** with low percent *ee* (~ 35%), which was linked to the low stereoselectivity of both mutants in the reduction of phenylacetone.^[12c,14] Furthermore, an excellent stereoinversion of (*R*)-4-(4'-hydroxyphenyl)-2-butanol [(*R*)-**1d**] to (*S*)-**1d** [from >99% *ee* of (*R*)-**1d** to >99% *ee* of (*S*)-**1d**] was achieved under identical conditions using W110G TeSADH. However, moderately low percent *ee* of (*S*)-**1d** was obtained when W110A TeSADH was used in the stereoinversion of (*R*)-**1d** (Table 2), which was attributed to incomplete oxidation of the latter (Table 1, entry 12) that led to a lower *ee* of the recovered alcohol. To the contrary, the presence of methoxy group at the *para* position of benzylacetone significantly reduced the stereospecificity of W110A TeSADH to oxidize (*R*)-**1e**, allowing its complete oxidation to the corresponding ketone. The low *ee* of the recovered alcohol [82% *ee* (*S*)-**1e**] using W110A TeSADH was considered due to the relatively low enantioselectivity of the reduction reaction of the intermediate ketone **2e**, compared to that of **2d**.^[12b]

Table 2. Stereoconversion of phenyl-ring-containing enantiopure *R* alcohols catalyzed by X TeSADH (X = W110A or W110G).^[a]

Substrate	R	X	nb (%)	ee (%) ^[b]
(<i>R</i>)- 1a	C ₆ H ₅ CH ₂ CH ₂	W110G	4	80(<i>S</i>)
		W110A	<0.5	86(<i>S</i>)
(<i>R</i>)- 1b	C ₆ H ₅ CH ₂	W110G	<0.5	33(<i>S</i>)
		W110A	<0.5	35(<i>S</i>)
(<i>R</i>)- 1d	<i>p</i> -HO-C ₆ H ₄ CH ₂ CH ₂	W110G	<0.5	>99(<i>S</i>)
		W110A	<0.5	64(<i>S</i>)
(<i>R</i>)- 1e	<i>p</i> -MeO-C ₆ H ₄ CH ₂ CH ₂	W110A	<0.5	82(<i>S</i>)

[a] Unless stated, oxidation reactions were performed using 0.03 mmol (*R*)-**1a-e**, 0.4 mg mutant TeSADH, and 1.0 mg NADP⁺ in 1.0 mL Tris-HCl buffer solution (50 mM, pH 8.0) containing 3% acetone (v/v), at 50 °C with shaking at 180 rpm for 36 h; reduction reactions were performed using 30% 2-propanol (v/v) and additional 0.2 mg mutant TeSADH and 1.0 mg NADP⁺ at 50 °C with shaking at 180 rpm for 24 h. [b] The % *ee* of the acetate ester derivative of the product was determined using GC with a chiral stationary phase.

For practical applications, we next employed our stereoconversion approach on a larger scale using 0.2 mmol of the alcohol substrates to a final concentration of 20 mM. The oxidation and reduction reactions were conducted in Tris-HCl buffer solution containing acetone (2%, v/v) and 2-propanol (20%, v/v), respectively. In this case, the stereoconversion of (*R*)-**1a** led to the recovery of more than 95% of (*S*)-**1a** (70% *ee*) whereas stereoconversion of (*R*)-**1d** resulted in >99% recovery of (*S*)-**1d**, known as (*S*)-rhododendrol, in >99% *ee*. (*S*)-Rhododendrol is a naturally occurring alcohol that is used to inhibit melanin in skin-lightening cosmetics^[15] and to prevent hepatotoxicity.^[16]

To the best of our knowledge, this is the first report for a stereoconversion of enantiopure alcohols that uses a single enzyme. Extensive mutations of TeSADH and search for other enzymes with tunable stereoselectivity is essential for the utilization of this method in organic synthesis.

Conclusion

Stereoinversion of *R*-configured alcohol is accomplished using a single mutant of TeSADH. This approach relies on selectivity mistakes wherein site expansion of the larger pocket at the active site of TeSADH, a Prelog enzyme, allowed the recognition of the *R* enantiomer in the oxidation reaction followed by the (*S*)-selective reduction of the resultant ketone under stereoselective conditions. This approach eliminates the need to isolate ketone intermediate and thereby allows the recovery of the *S*-configured alcohols with high enantioselectivity. The stereoselectivity of the oxidation and the reduction steps are easily controlled by varying the concentration of the cosubstrates acetone and 2-propanol, respectively. This approach represents a greener alternative for the rigorous Mitsunobu stereoinversion of alcohols.

Supporting Information Summary

Experimental procedures and gas chromatograms.

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Keywords: Alcohol dehydrogenase, redox reactions, stereoinversion, stereoselectivity

References

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- [1] Y. K. Agrawal, H. G. Bhatt, H. G. Raval, P. M. Oza, P. J. Gorgoi, *Mini-Rev. Med. Chem.* **2007**, *7*, 451–460.
- [2] (a) E. Fogassy, M. Nogradi, D. Kozma, G. Egri, E. Palovics, V. Kiss, *Org. Biomol. Chem.* **2006**, *4*, 3011–3030; (b) A. Ghanem, H. Y. Aboul-enein, *Chirality* **2005**, *17*, 1–15.
- [3] (a) M. Rachwalski, N. Vermue, F. P. J. T. Rutjes, *Chem. Soc. Rev.* **2013**, *42*, 9268–9282; (b) J. H. Lee, K. Han, M.-J. Kim, J. Park, *Eur. J. Org. Chem.* **2010**, 999–1015; (c) H. Pellissier, *Tetrahedron* **2011**, *67*, 3769–3802; (d) O. Verho, J.-E. Bäckvall, *J. Am. Chem. Soc.* **2015**, *137*, 3996–4009.
- [4] (a) C. V. Voss, C. C. Gruber, K. Faber, T. Knaus, P. Macheroux, W. Kroutil, *J. Am. Chem. Soc.* **2008**, *130*, 13969–13972; (b) C. V. Voss, C. C. Gruber, W. Kroutil, *Angew. Chem. Int. Ed.* **2008**, *47*, 741–745; *Angew. Chem.* **2008**, *120*, 753–757; (c) A. Diaz-Rodriguez, N. Rios-Lombardia, J. H. Sattler, I. Lavandera, V. Gotor-Fernandez, W. Kroutil, V. Gotor, *Catal. Sci. Technol.* **2015**, *5*, 1443–1446.
- [5] (a) T. Cazetta, P. J. S. Moran, A. R. Rodrigues, *J. Mol. Catal. B: Enzym.* **2014**, *109*, 178–183; (b) R. Sungür, B. Lurçi, E. Bayraktar, U. Mehmetoğlu, A. S. Demir, *Artif. Cells, Blood Substitutes, Biotechnol.* **2011**, *39*, 162–168; (c) M. C. Fragnelli, P. Hoyos, D. Romano, R. Gandolfi, A. R. Alcantara, F. Molinari, *Tetrahedron* **2012**, *68*, 523–528; (d) Y.-P. Xu, Y.-G. Zheng, Y.-Q. Zhang, J.-L. Sun, Z.-Q. Liu, Y.-C. Shen, *Chem. Commun.* **2013**, *49*, 10706–10708; (e) T. Saravanan, S. Jana, A. Chadha, *Org. Biomol. Chem.* **2014**, *12*, 4682–4690.
- [6] O. Mitsunobu, M. Yamada, *Bull. Chem. Soc. Jpn.* **1967**, *40*, 2380–2382.
- [7] K. C. K. Swamy, N. N. B. Kumar, E. Balaraman, K. V. P. P. Kumar, *Chem. Rev.* **2009**, *109*, 2551–2651.
- [8] J. S. Carey, D. Laffan, C. Thomson, M. T. Williams, *Org. Biomol. Chem.* **2006**, *4*, 2337–2347.
- [9] C. E. Paul, I. Lavandera, V. Gotor-Fernández, W. Kroutil, V. Gotor, *ChemCatChem* **2013**, *5*, 3875–3881.
- [10] I. Karume, M. Takahashi, S. M. Hamdan, M. M. Musa, *ChemCatChem* **2016**, *8*, 1459–1463.
- [11] V. Prelog, *Pure Appl. Chem.* **1964**, *9*, 119–130.

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- [12] (a) O. Bsharat, M. M. Musa, C. Vieille, S. A. Oladepo, M. Takahashi, S. M. Hamdan, *ChemCatChem* **2017**, *9*, 1487–1493; (b) M. M. Musa, O. Bsharat, I. Karume, C. Vieille, M. Takahashi, S. Hamdan, *Eur. J. Org. Chem.* **2018**, 798–805; (c) M. M. Musa, K. I. Ziegelmann-Fjeld, C. Vieille, J. G. Zeikus, R. S. Phillips, *J. Org. Chem.* **2007**, *72*, 30–34.
- [13] (a) M. M. Musa, R. S. Phillips, M. Laivenieks, C. Vieille, M. Takahashi, S. M. Hamdan, *Org. Biomol. Chem.* **2013**, *11*, 2911–2915; (b) M. M. Musa, J. M. Patel, C. M. Nealon, C. S. Kim, R. S. Phillips, I. Karume, *J. Mol. Catal. B: Enzym.* **2015**, *115*, 155–159.
- [14] J. M. Patel, M. M. Musa, L. Rodriguez, D. A. Sutton, V. Popik, R. S. Phillips, *Org. Biomol. Chem.* **2014**, *12*, 5905–5910.
- [15] Y. Yuasa, S. Shibuya, Y. Yuasa, *Synth. Commun.* **2003**, *33*, 1469–1475.
- [16] T. Fujita, *Phytochemistry* **1995**, *39*, 1085–1089.

Table of content Figure:

A stereoinversion approach of *R*-configured secondary alcohols catalyzed by a single alcohol dehydrogenase is achieved through a non-stereoselective oxidation to the corresponding ketones, followed by a stereoselective reduction catalyzed by the same alcohol dehydrogenase to produce the *S*-configures alcohols. This single enzymatic stereoinversion approach presents a biocatalytic alternative to the laborious Mitsunobu inversion reaction.