Pure Appl. Chem., Vol. 82, No. 1, pp. 117–128, 2010.
doi:10.1351/PAC-CON-09-01-03

How carbonyl reductases control stereoselectivity: Approaching the goal of rational design*

Dunming Zhu[1,‡] and Ling Hua[2]

[1]State Engineering Laboratory for Industrial Enzymes, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, China; [2]China Research Center, Genencor International, A Danisco Division, Shanghai 200335, China

Abstract: Although "Prelog's rule" and "two hydrophobic binding pockets" model have been used to predict and explain the stereoselectivity of enzymatic ketone reduction, the molecular basis of stereorecognition by carbonyl reductases has not been well understood. The stereoselectivity is not only determined by the structures of enzymes and substrates, but also affected by the reaction conditions such as temperature and reaction medium. Structural analysis coupled with site-directed mutagenesis of stereocomplementary carbonyl reductases readily reveals the key elements of controlling stereoselectivity in these enzymes. In our studies, enzyme-substrate docking and molecular modeling have been engaged to understand the enantioselectivity diversity of the carbonyl reductase from *Sporobolomyces salmonicolor* (SSCR), and to guide site-saturation mutagenesis for altering the enantioselectivity of this enzyme. These studies provide valuable information for our understanding of how the residues involved in substrate binding affect the orientation of bound substrate, and thus control the reaction stereoselectivity. The in silico docking-guided semi-rational approach should be a useful methodology for discovery of new carbonyl reductases.

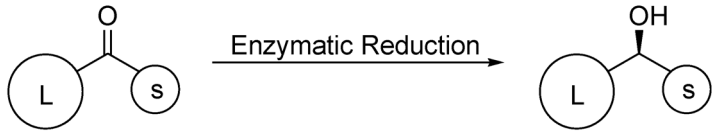
Keywords: carbonyl reductase; enzyme-substrate docking; ketone reduction; site-directed mutagenesis; stereoselectivity.

INTRODUCTION

Optically active alcohols are key building blocks in the preparation of high-value fine chemicals. A straightforward approach to access these enantiomerically pure alcohols is the reduction of the prochiral ketones. The ketone reduction can be conducted biocatalytically or chemically. Biocatalytic ketone reduction possesses unparalleled chemo-, regio-, and stereoselectivity as well as environmentally benign reaction conditions, thus its application in the fine chemical industry has been expanding rapidly to address the industrial challenges of sustainability and profitability [1,2]. Carbonyl reductases (also known as ketoreductases or alcohol dehydrogenases) catalyze hydride transfer from cofactor NAD(P)H to prochiral ketone to afford either *R*- or *S*-enantiomers. Although researchers have been trying to understand the origin of the stereoselectivity since the discovery of biocatalytic ketone reduction, it is still a puzzling question and the molecular basis of stereorecognition by carbonyl reductases is not well un-

*Paper based on a presentation at the 13th International Biotechnology Symposium (IBS 2008): "Biotechnology for the Sustainability of Human Society", 12–17 October 2008, Dalian, China. Other presentations are published in this issue, pp. 1–347.
‡Corresponding author

derstood up to now. In the early 1960s, from the stereochemistry of microbial reduction of a great number of ketones with the culture of *Curvularia falcata*, Prelog proposed a simple model (Scheme 1) to predict the product stereochemistry based on the steric requirements of the substrates [3]. "Prelog's rule" shows that the hydride was delivered from the *Re*-face of a prochiral ketone to form the (*S*)-configurated product alcohol. He also used "diamond lattice sections" analysis to show that the non-bonded interaction of substrate and cofactor in the hydride transfer transition state played an important role in determining the stereoselectivity. The majority of known carbonyl reductases used for the asymmetric ketone reduction follow "Prelog's rule", but more and more enzymes have been found to catalyze ketone reduction to furnish anti-Prelog's rule product alcohols.



Scheme 1 "Prelog's rule" for enzymatic ketone reduction.

While an alcohol dehydrogenase from *Thermoanaerobacter brockii* (TBADH) obeyed "Prelog's rule" to afford (*S*)-alcohols when larger aliphatic ketones than 2-pentanone were used as the substrates, (*R*)-alcohols were obtained for the reduction of small aliphatic ketones such as 2-butanone [4]. Keinan et al. proposed that the enzyme has two hydrophobic sites, or pockets of different size, with the smaller one having greater affinity toward the alkyl groups (Fig. 1). For 2-butanone, the ethyl group can enter the smaller site because of its higher affinity to give the (*R*)-2-butanol as the major product. For the larger ketones, the larger alkyl group was forced to enter the larger site with the smaller alkyl group being accommodated into the smaller pocket to furnish the (*S*)-configurated products.

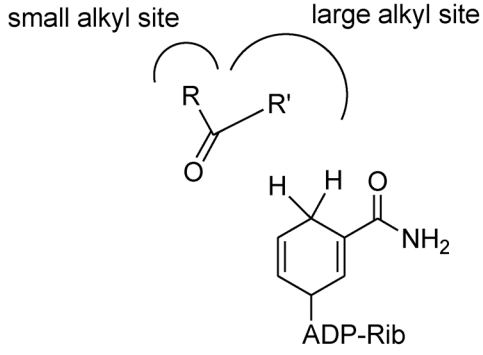


Fig. 1 Proposed active sites of alcohol dehydrogenase from TBADH.

In this overview, the recent advances in some aspects concerning the stereoselectivity of carbonyl reductases will be presented, including the temperature and solvent effects on enzyme enantioselectivity, stereocomplementary carbonyl reductases, and our recent results on the studies of a carbonyl reductase from *Sporobolomyces salmonicolor* (SSCR), in an attempt to provide some insights into the molecular basis for the mechanism of carbonyl reductases controlling their stereoselectivity, and to demonstrate that the structure-guided mutagenesis is a useful methodology to tune the enantioselectivity of these carbonyl reductases.

TEMPERATURE AND SOLVENT EFFECTS ON ENZYME ENANTIOSELECTIVITY

The previous empirical models imply that the enantioselectivity of enzymatic ketone reduction is dependent on the steric requirement and van der Waals forces of substrate binding which are contained in the activation enthalpy. For some carbonyl reductases, the enantioselectivity is temperature-dependent. For example, a secondary alcohol dehydrogenase from *Thermoanaerobacter ethanolicus* (SADH) and its mutant S39T show an increase in (*R*)-specificity with increasing temperature [5,6]. Phillips et al. analyzed the temperature effect on stereoselectivity of the reverse oxidation using both enantiomers of 2-butanol, 2-pentanol, and 2-hexanol as substrates according to equation [$-RT \ln E = \Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$, where E is defined as $(k_{cat}/K_m)_R/(k_{cat}/K_m)_S$], and found that the temperature-dependence is due to a relatively large activation entropy difference, $\Delta\Delta S^{\ddagger}$, between the (*R*)- and (*S*)-enantiomers, favoring the formation of the (*R*)-enantiomer. Surprisingly, the stereoselectivity of mutant C295A SADH shows no significant temperature dependence [7]. Modeling studies using the X-ray structure of highly homologous (96 % identity) alcohol dehydrogenase from TBADH indicates that Cys295 is located in the small alkyl binding pocket in Fig. 1. The SH of Cys295 may interact with bound ordered water molecule in the small alkyl site as in the structure of TBADH. This bound water molecule could be selectively expelled from the small alkyl binding pocket upon binding of a large substituent into the small alkyl pocket. As reaction temperature increases, the bound water molecule can be expelled more easily, thus favoring formation (*R*)-enantiomer at higher temperature. Replacement of Cys295 with Ala excludes this bound water in the small binding pocket and the corresponding entropy change during reaction. As such, it is possible that the Cys295-bound water molecule is responsible for the large activation entropy difference, $\Delta\Delta S^{\ddagger}$, thus at least partially determines the temperature effect on the enzyme's enantiospecificity [7–9].

Actually, it has been observed that water activity affects the stereoselectivity of ketone reduction in hexane catalyzed by TBADH [4,10]. For the reduction of 2-pentanone, the *S*/*R*-enantiomer ratio increases from 2.6 to 4.6 when the water activity in hexane increases from 0.53 to 0.97. As the water amount increases so that the solubility limit is exceeded, a distinct aqueous phase is formed and the reaction medium becomes biphasic. The effect on enantioselectivity of changing reaction medium from hexane containing 2.5 % (v/v) to pure water is negligible because of the minimal related change of water content in the active site [10]. The enantioselectivity of TBADH also remains unchanged in water-miscible organic solvents [11]. A few additional examples of tuning enantioselectivity of ketone reduction by changing the reaction medium have been reported for the alcohol dehydrogenases from *Thermoanaerobacterium* sp. KET4B1 [12], *Lactobacillus brevis* [13], and W110A mutant from *Thermoanaerobacter ethanolicus* [14,15].

STEREOCOMPLEMENTARY CARBONYL REDUCTASES

In recent years, the crystal structure and modeling studies together with site-directed mutagenesis provide some insights into the structural basis for the stereoselectivity of enzyme-catalyzed ketone reduction, especially where a pair of stereocomplementary carbonyl reductases exists in the same metabolic pathway. NADPH-dependent tropinone reductase I (TR-I) and II (TR-II) are key enzymes in the biosynthetic pathway of tropane alkaloids of medicinal importance such as hyoscyamine and scopolamine. TR-I catalyzes the reduction of 3-carbonyl group of tropinone to an α-hydroxyl group affording tropine as the product, while TR-II reduces the same substrate tropinone to ψ-tropine with a β-hydroxyl group [16]. These two enzymes from *Datura stramonium* share 64 % identical amino acid residues, have similar K_m values for NADPH, and catalyze the *pro-S* hydride transfer to tropinone. X-ray crystallographic analysis of TR-I and TR-II has revealed their highly conserved overall folding [17]. Molecular modeling has suggested that the substrate tropinone binds with the putative substrate binding site in different orientations toward the *pro-S* hydride of NADPH, leading to the distinct stereospecificities of TR-I and TR-II. This has been confirmed by the crystal structure analysis of tropinone reductase-II complexed

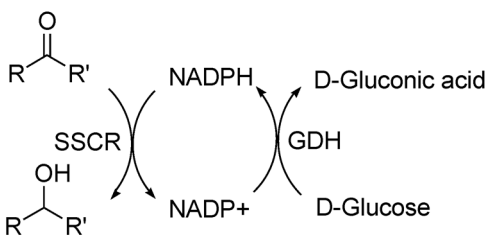
with $NADP^+$ and pseudotropine, in which the substrate binding site is mainly constituted by hydrophobic residues and the van der Waals interactions between the substrate (ψ-tropine or tropinone) and the binding site likely contribute to the correct substrate orientation [18]. Substitution of five amino acid residues of the substrate binding site from one tropinone reductase with those found in the corresponding positions of the other TR resulted in an almost complete reversal of the stereospecificity. This demonstrates that these key residues indeed control the binding orientation of tropinone in the binding site, thus dictating the different stereospecificities of TR-I and TR-II [19].

Another known pair of stereocomplementary reductases is (*R*)-hydroxypropyl-coenzyme M dehydrogenase (*R*-HPCDH) and (*S*)-hydroxypropyl-coenzyme M dehydrogenase (*S*-HPCDH), which act in concert in epoxide metabolic pathway of *Xanthobacter autotrophicus* Py2 [20]. The crystal structure analysis of *R*-HPCDH together with biochemical studies suggests that the substrate sulfonate binding is the key to align the substrate in the correct orientation in the active site for hydride transfer. Comparison of the structure of *R*-HPCDH with the homology model of *S*-HPCDH reveals the differences at the substrate binding sites of these two enzymes, strongly supporting sulfonate binding as the key for the stereodiscrimination [21]. The ketoreductase domains $eryKR_1$ and $eryKR_2$ from the erythromycin-producing polyketide synthase (PKS) also shows opposite stereospecificity in the reduction of 3-ketoacyl-thioester intermediates. A double mutation (F141W, P144G) at the active site of eryKR1 domain switches the product of reduction of (2*R*,*S*)-2-methyl-3-oxopentanoic acid *N*-acetylcysteamine thioester from (2*S*,3*R*)-3-hydroxy-2-methylpentanoic *N*-acetylcysteamine thioester to the (2*S*,3*S*) isomer [22,23].

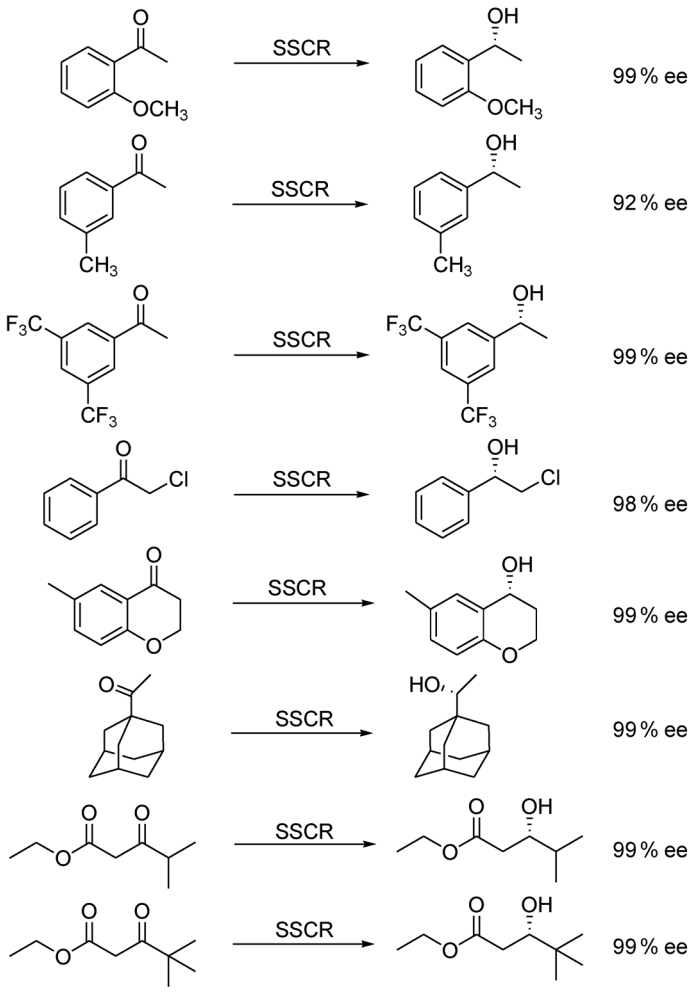
CARBONYL REDUCTASE FROM SSCR

In contrast to these stereocomplementary reductases, in which comparative studies readily reveal the key elements of determining stereoselectivity, the majority of carbonyl reductases have been much less understood in their control of stereoselectivity. Since protein structure information provides valuable insights into the structure–function relationship of an enzyme, we have studied the activity and enantioselectivity of a carbonyl reductase (SSCR) from *Sporobolomyces salmonicolor* AKU 4429, of which the X-ray structures and its complex with a coenzyme (NADPH) have been determined [24,25], toward the reduction of a variety of ketones with diverse structures. SSCR belongs to the short-chain dehydrogenase/reductase family. The structure of NADPH-binding domain and the interaction between the enzyme and NADPH are very similar to those found in other structure-solved enzymes of same family, while the structure of substrate-binding domain is unique. This unique substrate-binding domain suggests that SSCR might possess an unusual substrate specificity and stereoselectivity. Indeed, SSCR shows an unusually broad substrate range including aliphatic, aromatic ketones, α- and β-ketoesters, and sterically bulky aryl alkyl ketones and diaryl ketones [26,27].

The SSCR-catalyzed reduction of ketones was carried out using an NADPH regeneration system consisting of D-glucose dehydrogenase (GDH) and D-glucose as shown in Scheme 2. This carbonyl re-



Scheme 2 Ketone reduction catalyzed by SSCR with a NADPH regeneration system consisting of GDH and D-glucose.



Scheme 3 Selected examples of SSCR-catalyzed reduction of a diversity of ketones.

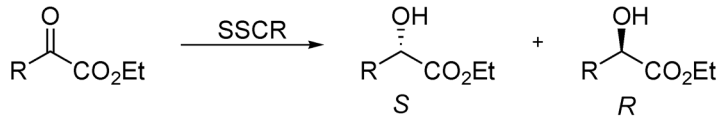
ductase catalyzes reduction of a diversity of ketones to furnish the corresponding alcohols with excellent enantiomeric purity. A few examples are given in Scheme 3.

Reduction of α-ketoesters

It is interesting to observe that aromatic α-ketoesters are reduced by SSCR to (*S*)-enantiomers, while the reduction of aliphatic counterparts affords (*R*)-enantiomers with excellent ee values (Table 1) [26]. To gain insight on how the R group affects the configuration of the product alcohols, enzyme-substrate docking was performed with ethyl phenylglyoxylate and ethyl 3,3-dimethyl-2-oxobutyrate. In the lowest-energy docked conformations of these two substrates into the enzyme active site, the carbonyl carbon is in position proximal to the C4 atom of the nicotinamide ring of the cofactor NADPH (Fig. 2), facilitating the hydride transfer from NADPH to the ketone. As shown in Fig. 2, the NMN ring of the cofactor is located at the *pro-S* face of ethyl phenylglyoxylate (A), but at the *pro-R* face of ethyl 3,3-dimethyl-2-oxobutyrate (B), the hydride transfer from NADPH to the carbonyl group thus occurs from different faces for aromatic and aliphatic α-ketoesters. The orientations of the substrates in the active site revealed by the enzyme-substrate docking study are consistent with the experimental observations

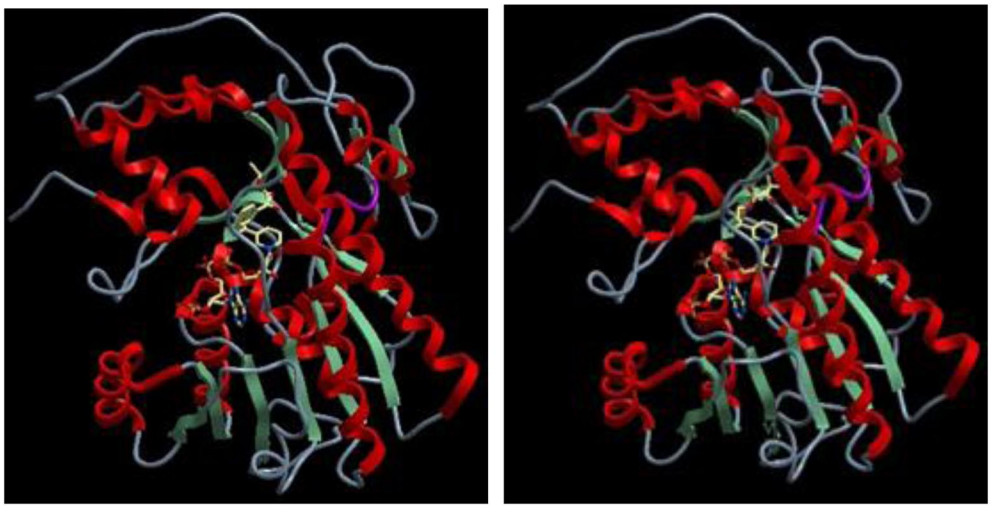
that ethyl (*S*)-2-hydroxy-2-phenylacetate and ethyl (*R*)-3,3-dimethyl-2-hydroxybutyrate are obtained, respectively.

Table 1 SSCR-catalyzed reduction of α-ketoesters.



R	Specific activity[a]	ee (%)	Absolute configuration
Phenyl	6.64	99	*S*
4-Cyanophenyl	2.28	82	*S*
4-Fluorophenyl	5.07	74	*S*
4-Chlorophenyl	6.36	63	*S*
4-Bromophenyl	1.40	56	*S*
4-Methylphenyl	1.20	88	*S*
3,5-Difluorophenyl	5.73	43	*S*
iso-Propyl	17.54	99	*R*
tert-Butyl	5.56	99	*R*

[a]The specific activity was defined as μmol min^{-1} mg^{-1}.



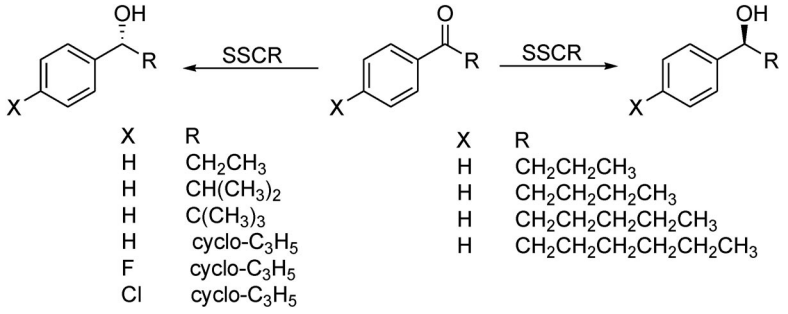
(A) (B)

Fig. 2 Lowest-energy docked conformations of ethyl phenylglyoxylate (A) and ethyl 3,3-dimethyl-2-oxobutyrate (B) into the enzyme active site.

Reduction of aryl alkyl ketones

Similarly, an unusual alkyl chain-induced enantiopreference reversal has been observed in the reduction of aryl alkyl ketones with linear alkyl groups as shown in Scheme 4 [27]. SSCR effectively catalyzes the enantioselective reduction of aryl alkyl ketones having a number of diverse alkyl groups. The absolute configuration of the major product is switched from (*R*) to (*S*) when the length of the linear alkyl chain increases from 2 to 3 carbon atoms. The enantiopreference remains unchanged and enantioselec-

tivity is dramatically enhanced when the alkyl group becomes branched (e.g., *iso*-, *tert*-, and *cyclo*-propyl, Table 2).



Scheme 4 SSCR-catalyzed reduction of aryl alkyl ketones.

Table 2 Interaction energies between substrate conformers and enzyme[a] and the enantiomeric excess of the major reduction products.

R in ketone	X in ketone	$E_{int.}$ (kcal/mol) "left" conformer	$E_{int.}$ (kcal/mol) "right" conformer	ee (absolute configuration)
CH_3	H	–19.3	–15.2	42 (R)
CH_2CH_3	H	–21.0	–20.6	28 (R)
$(CH_2)_2CH_3$	H	–21.3	–23.2	88 (S)
$(CH_2)_3CH_3$	H	–21.3	–21.8	87 (S)
$(CH_2)_4CH_3$	H	–21.2	–24.2	34 (S)
$(CH_2)_5CH_3$	H	–25.5	–25.9	27 (S)
$CH(CH_3)_2$	H	–22.7	–19.6	98 (R)
$C(CH_3)_3$	H	–22.5	–19.5	98 (R)
Cyclo-C_3H_5	H	–22.1	–18.6	96 (R)
Cyclo-C_3H_5	F	–21.0	–20.2	98 (R)
Cyclo-C_3H_5	Cl	–21.6	–19.2	98 (R)

[a]Calculated on fully geometry-optimized models of ketone-bound 1Y1P after molecular dynamics-based conformation search. See Scheme 4 for the definition of R and X. The enantiomeric excess is given in percent.

Enzyme-substrate docking and modeling studies have found that the lowest-energy conformations of the aryl alkyl ketones in the enzyme active site are consistent with these intriguing experimental results [28]. Favorable docking conformations for aryl alkyl ketones fall into two low-energy ensembles, in which the nicotinamide reductant is positioned at either the *Si*- or *Re*-face of the ketone leading to (*R*)- or (*S*)-alcohol (Fig. 3). Geometry optimizations of the lowest-energy "left" (*Si*-face) and "right" (*Re*-face) conformations obtained from the docking simulations were carried out, and the interaction energies (E_{int}) between the substrates and the active site in the Michaelis–Menten complexes were calculated using the Amber94 force field (Table 2). Interaction energies (E_{int}) are ≈ –20 kcal/mol, and increase slightly as the size of the alkyl group of the ketone becomes larger. The simulations suggest that the majority (~85 %) of the stabilization energy of the ketone in the Michaelis–Menten complexes is due to hydrophobic/van der Waals forces with a lesser contribution from electrostatic interactions [28].

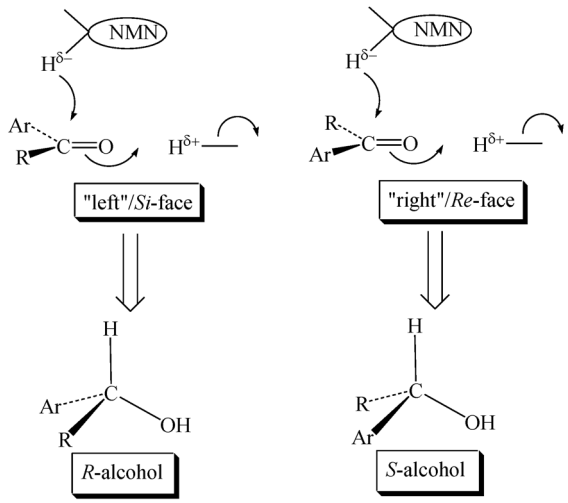


Fig. 3 Receptor site model for NADPH-dependent carbonyl reductase.

As shown in Table 2, the interaction energies between the "left" conformer and the enzyme active site in the Michaelis–Menten complexes are lower than that of the "right" conformer for ketones PhC(O)R, where R = methyl, ethyl, and branched alkyl groups such as *iso*-propyl, *tert*-butyl, and *cyclo*-propyl. In the "left" conformer, the hydride is transferred from the cofactor to the carbonyl group at the *Si*-face, leading to the (*R*)-enantiomer. As such, the (*R*)-enantiomer was obtained as the major product for these ketones. For the other aryl alkyl ketones in Table 2, lower interaction energies (E_{int}) with the active site are obtained for the "right" conformers, and in these cases the reduction of ketones gives (*S*)-enantiomer as the major product. Therefore, there is a correlation between the experimentally observed major enantiomer of product alcohols and the conformer having the most stable interaction energy with the receptor site (E_{int}) in the Michaelis–Menten complexes. The enantioselectivity should be quantitatively related to the activation energy difference between the two transition states leading to (*R*)- and (*S*)-enantiomers, respectively, according to equation, $-RT \ln E = \Delta\Delta G^{\ddagger} = \Delta\Delta H^{\ddagger} - T\Delta\Delta S^{\ddagger}$, where E is defined as $(k_{cat}/K_m)_R/(k_{cat}/K_m)_S$. The interaction energies (E_{int}) for the substrates with the active site in Table 2 correspond to the energies of Michaelis–Menten complexes. Since the difference in interaction energies ΔE_{int} is not equal to $\Delta\Delta G^{\ddagger}$ as shown in the reaction coordinate (Fig. 4), it is expected that there is no quantitative relationship between the enantiomeric values and the binding energy difference (ΔE_{int}) of the two low-energy docking conformers. However, it seems that the lower-binding energy comformer in the Michaelis–Menten complex is related to a lower activation energy, thus leading to the major enantiomer of product alcohol. As such, the sign of binding energy difference (ΔE_{int}) of the two low-energy docking conformers may be used to predict the major enantiomer of the product alcohols.

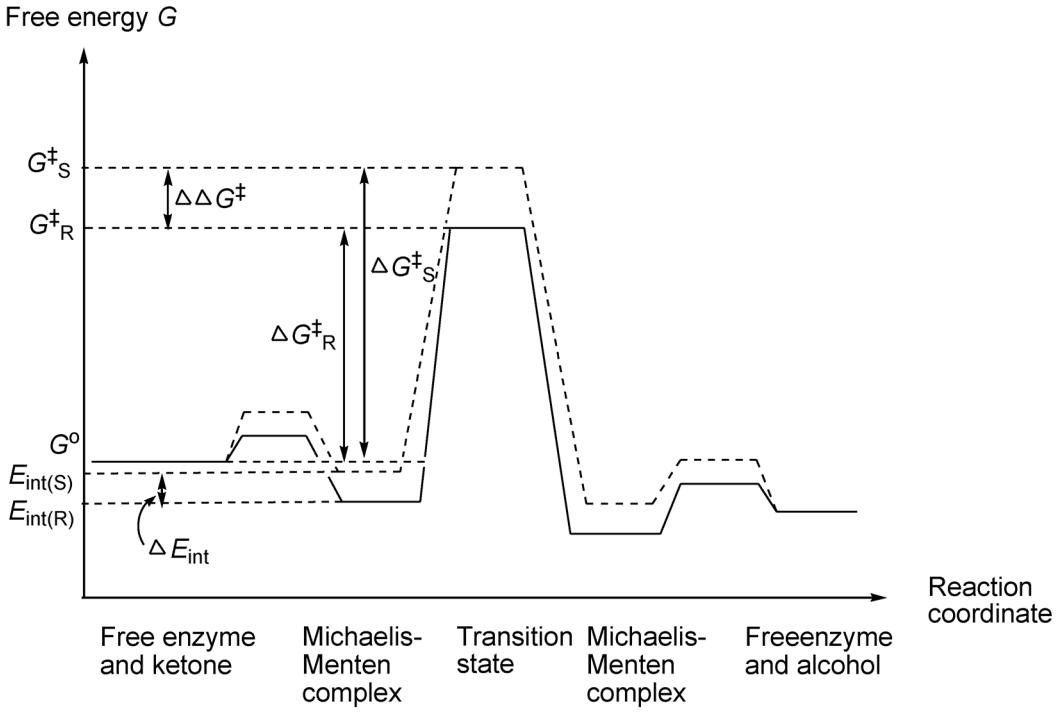


Fig. 4 Reaction coordinate of enzyme-catalyzed ketone reduction.

Enzyme-substrate docking-guided point mutation leading to inverted enantioselectivity

The enzyme-substrate docking and modeling not only rationalizes the observed stereospecificity of SSCR-catalyzed reductions, but also provides a starting point for identifying key residues for site-directed mutations that can enhance selectivity and activity, as well as broaden the scope of possible substrates. While a variety of ketone substrates are reduced by SSCR to afford the alcohol products with excellent optical purity, the enantioselectivity for the reduction of *para*-substituted acetophenones is low (14–59 % ee). An initial substrate-enzyme docking study was performed with 4'-methoxyacetophenone as substrate. Two opposite conformations, which are energetically close to each other and yield (*S*)- or (*R*)-enantiomer of product alcohol, respectively, have been found in the high scoring docking conformations (Fig. 5). This is qualitatively consistent with the low observed enantioselectivity.

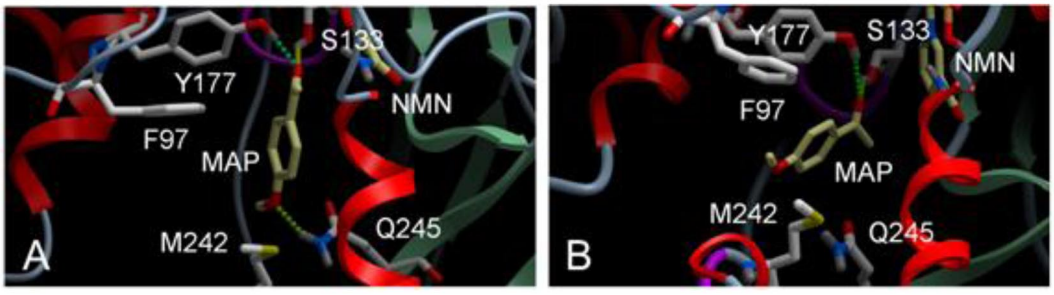
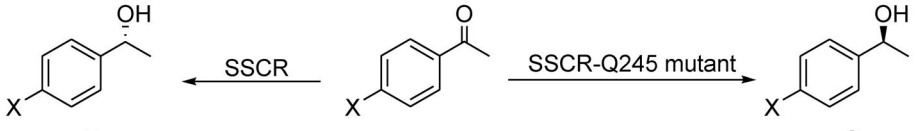


Fig. 5 The substrate 4'-methoxyacetophenone docked into the active site of SSCR. Two energetically close docking conformations with (A) leading to (*S*)-enantiomer and (B) forming (*R*)-enantiomer.

In both conformations, the residues Q245 and M242 are in close proximity to the *para*-substituent of the acetophenones with significant hydrogen bonding or hydrophobic interactions. Such close interaction might be responsible for the observed low enantioselectivity in the reduction of *para*-substituted acetophenones. As such, mutation of the residues Q245 and M242 in the catalytic site might increase the energetic difference between these two conformations, thus improving the enzyme enantioselectivity. To test this hypothesis, a focused library of mutants was created by saturation mutagenesis of the residue Q245 in the SSCR catalytic cavity. The resulting mutant library was screened using 4'-methoxyacetophenone as substrate. Three mutant enzymes (Q245H, Q245P, and Q245L) have been found to catalyze the reductions of *para*-substituted acetophenones to give (*S*)-configurated chiral alcohols in greater than 90 % ee, but the unsubstituted acetophenone was reduced in a relatively lower enantioselectivity (Table 3). Compared to the wild-type SSCR, these mutant SSCR enzymes exhibit an inverted enantiopreference and enhanced enantioselectivity. It is very remarkable that a single mutation results in such dramatic change in the enantioselectivity, indicating that the residue 245 in the catalytic cavity really plays a critical role in determining the enantioselectivity for the reduction of the *para*-substituted acetophenones.

Table 3 Enantiomeric excess values and absolute configurations of the reduction product alcohols of *para*-substituted acetophenones catalyzed by SSCR and its Q245 mutants.



X	SSCR-WT	Q245H	Q245P	Q245L
4'-H	42 (*R*)	78 (*S*)	64 (*S*)	82 (*S*)
4'-F	46 (*R*)	92 (*S*)	90 (*S*)	93 (*S*)
4'-Cl	14 (*R*)	90 (*S*)	96 (*S*)	96 (*S*)
4'-Br	42 (*R*)	92 (*S*)	98 (*S*)	97 (*S*)
4'-CH_3	59 (*R*)	95 (*S*)	96 (*S*)	95 (*S*)
4'-OCH_3	57 (*R*)	79 (*S*)	98 (*S*)	96 (*S*)
4'-$C(CH_3)_3$	31 (*R*)	96 (*S*)	99 (*S*)	99 (*S*)

A few mutant enzymes with mutation at M242 have also been found to switch the major reduction product alcohols of *para*-substituted acetophenones from (*R*)- to (*S*)-enantiomer, but the ee values of the products are lower than 90 % in most cases. It is clear that residue 242 in the catalytic site is also important in determining the enantioselectivity of these ketone reductions, but to a less extent than residue 245.

In order to gain insight into how the single mutation at residue 245 results in such a drastic change of enantioselectivity for the reduction of *para*-substituted acetophenones, in silico mutagenesis of Q245 to H and docking of 4'-methoxyacetophenone to the mutant Q245H were performed. Contrast to docking with the wild-type SSCR enzyme, 4'-methoxyacetophenone adopts one energetically preferred conformation, which is facilitated by the hydrophobic contacts between the 4'-methoxy group and the mutated residue H245. As shown in Fig. 6, the NMN ring of the cofactor is optimally positioned at the *Re*-face of the substrate, and the carbonyl oxygen atom of the substrate forms a hydrogen bond with Y177 or S133. As such, protonation and subsequent hydride attack lead to the corresponding (*S*)-1-(4'-methoxyphenyl)ethanol. Other *para*-substituted acetophenone substrates have also docked into the mutants Q245H, Q245P, and Q245L. It has been found that the substrates also adopt the energetically preferred *Re*-face conformation, in agreement with the experimental observation. The site-

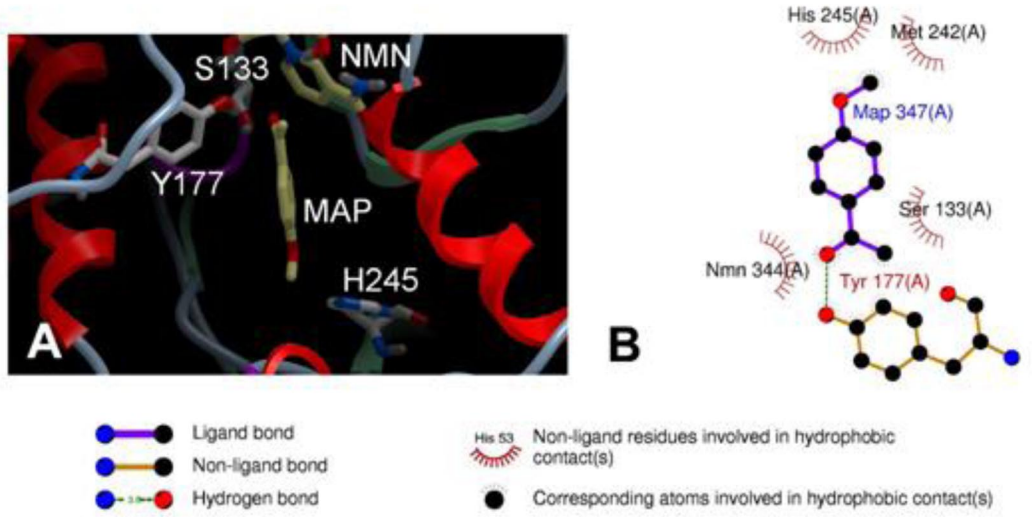


Fig. 6 The substrate 4'-methoxyacetophenone docked to the Q245H mutant of SSCR. (A) The energetically preferred conformation. (B) Ligplot showing significant binding interactions between the flexibly docked substrate and SSCR.

mutagenesis together with enzyme substrate docking studies confirms the important role of the residues Q245 and M242 (in close contact with the *para*-substituent) in determining the enzyme's enantioselectivity toward the reduction of *para*-substituted acetophenones.

CONCLUSION

These studies have shown that the interactions between the ketone and the residues in substrate binding site (mainly hydrophobic/van der Waals forces, sometimes electrostatic interactions or hydrogen bonding) determine the orientation of bound substrate, and thus control the enzyme's stereoselectivity. The in silico docking-guided semi-rational approach has been shown to be a valuable methodology for discovery of new carbonyl reductases, where high-throughput methods for rapid screening of large numbers of mutants to determine enantioselectivity are difficult to implement [29].

ACKNOWLEDGMENTS

D.Z. thanks the Chinese Academy of Sciences for support from the Knowledge Innovation Program (Grant No. KSCX2-YW-G-031) and Tianjin Municipal Science & Technology Commission for financial support (Grant No. 09ZCKFSH01000).

REFERENCES

1. S. M. A. De Wildeman, T. Sonke, H. E. Schoemaker, O. May. *Acc. Chem. Res.* **40**, 1260 (2007).
2. J. C. Moore, D. J. Pollard, B. Kosjek, P. N. Devine. *Acc. Chem. Res.* **40**, 1412 (2007).
3. V. Prelog. *Pure Appl. Chem.* **9**, 119 (1964).
4. E. Keinan, E. K. Hafeli, K. K. Seth, R. Lamed. *J. Am. Chem. Soc*. **108**, 162 (1986).
5. V. T. Pham, R. S. Phillips. *J. Am. Chem. Soc*. **112**, 3629 (1990).
6. A. E. Tripp, D. S. Burdette, J. G. Zeikus, R. S. Phillips. *J. Am. Chem. Soc*. **120**, 5137 (1998).
7. C. Heiss, M. Laivenieks, J. G. Zeikus, R. S. Phillips. *J. Am. Chem. Soc*. **123**, 345 (2001).
8. R. S. Phillips. *J. Mol. Catal., B: Enzymatic* **19–20**, 103 (2002).

9. R. S. Phillips. *Can. J. Chem.* **80**, 680 (2002).
10. A. Jonsson, E. Wehtje, P. Adlercreutz, B. Mattiasson. *Biochim. Biophys. Acta* **1430**, 313 (1999).
11. L. Olofsson, I. A. Nicholls, S. Wikman. *Org. Biomol. Chem*. **3**, 750 (2005).
12. H. D. Simpson, D. A. Cowan. *Protein Peptide Lett*. **4**, 25 (1997).
13. J. Schumacher, M. Eckstein, U. Kragl. *Biotechnol. J.* **1**, 574 (2006).
14. M. M. Musa, K. I. Ziegelmann-Fjeld, C. Vieille, R. S. Phillips. *Org. Biomol. Chem.* **6**, 887 (2008).
15. M. M. Musa, K. I. Ziegelmann-Field, C. Vieille, J. G. Zeikus, R. S. Phillips. *Angew. Chem., Int. Ed.* **46**, 3091 (2007).
16. K. Nakajima, T. Hashimoto, Y. Yamada. *Proc. Natl. Acad. Sci. USA* **90**, 9591 (1993).
17. K. Nakajima, A. Yamashita, H. Akama, T. Nakatsu, H. Kato, T. Hashimoto, J. i. Oda, Y. Yamada. *ICR Annual Report* **5**, 44 (1999).
18. A. Yamashita, H. Kato, S. Wakatsuki, T. Tomizaki, T. Nakatsu, K. Nakajima, T. Hashimoto, Y. Yamada, J. i. Oda. *Biochemistry* **38**, 7630 (1999).
19. K. Nakajima, H. Kato, J. i. Oda, Y. Yamada, T. Hashimoto. *J. Biol. Chem*. **274**, 16563 (1999).
20. J. R. Allen, S. A. Ensign. *Biochemistry* **38**, 247 (1999).
21. A. M. Krishnakumar, B. P. Nocek, D. D. Clark, S. A. Ensign, J. W. Peters. *Biochemistry* **45**, 8831 (2006).
22. H. M. O'Hare, A. Baerga-Ortiz, B. Popovic, J. B. Spencer, P. F. Leadlay. *Chem. Biol.* **13**, 287 (2006).
23. A. Baerga-Ortiz, B. Popovic, A. P. Siskos, H. M. O'Hare, D. Spiteller, M. G. Williams, N. Campillo, J. B. Spencer, P. F. Leadlay. *Chem. Biol.* **13**, 277 (2006).
24. K. Kita, T. Fukura, K.-I. Nakase, K. Okamoto, H. Yanase, M. Kataoka, S. Shimizu. *Appl. Environ. Microbiol*. **65**, 5207 (1999).
25. S. Kamitori, A. Iguchi, A. Ohtaki, M. Yamada, K. Kita. *J. Mol. Biol.* **352**, 551 (2005).
26. D. Zhu, Y. Yang, J. D. Buynak, L. Hua. *Org. Biomol. Chem*. **4**, 2690 (2006).
27. D. Zhu, L. Hua. *J. Org. Chem.* **71**, 9484 (2006).
28. T. R. Cundari, A. Dinescu, D. Zhu, L. Hua. *J. Mol. Model*. **13**, 685 (2007).
29. M. D. Truppo, F. Escalettes, N. J. Turner. *Angew. Chem., Int. Ed*. **47**, 2639 (2008).

