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A molecular modeling study on the enantioselectivity of aryl alkyl ketone reductions by a NADPH-dependent carbonyl reductase

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Abstract Automated structural analysis of *Sporobolomyces salmonicolor* carbonyl reductase (**SSCR**) indicates that the two largest potential receptor sites are in the vicinity of the nicotinamide reductant. The largest receptor site is a scalene triangle with sides of ~8 Å by 9 Å by 13 Å, which is narrow in width; one corner is surrounded by hydrophilic residues that can favorably bond with the ketone oxygen. Docking aryl alkyl ketones shows a distinct preference for binding to the largest receptor site, and for conformations that place the carbonyl oxygen of the substrate in the hydrophilic corner of the largest receptor site. Favorable docking conformations for aryl alkyl ketones fall into two low-energy ensembles. These conformational ensembles are distinguished by the positions of the substituents, presenting either the *Si*-or *Re*-face of the ketone to the nicotinamide reductant. For the ketones investigated here, there is a correspondence between the major enantiomer of the alcohol obtained from the reduction of the ketone and the conformer found to have the most stable interaction energy with the receptor site in all cases. The receptor site modeling, docking simulations, molecular dynamics, and enzyme-substrate geometry optimizations lead to a model for understanding the enantioselectivity of this NADPH-dependent carbonyl reductase.

Keywords Molecular modeling · Ketone reduction · Enantioselectivity · Enzymatic reduction · Carbonyl reductase

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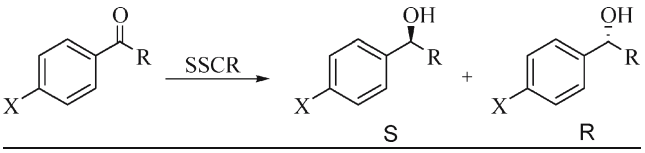
Introduction

Chiral alcohols are important as bioactive compounds or as precursors to such molecules. Therefore, the asymmetric reduction of ketones to their corresponding alcohols is becoming an increasingly important transformation in organic synthesis. Because of their environmentally benign reaction conditions and unparalleled chemo-, regio- and stereoselectivities, enzymatic reductions of ketones have attracted more and more attention [1]. Thus, there is a constant demand for efficient biocatalysts for this vital transformation, and many new biocatalytic systems both in whole cell and isolated enzyme forms have recently been developed [2–8]. As part of our effort to develop an effective ketoreductase tool-box of isolated enzymes which complement the currently available ones [9–11], a carbonyl reductase from *Sporobolomyces salmonicolor* (**SSCR**) was found to effectively catalyze the enantioselective reduction of various ketones having a number of diverse structures [12, 13]. Of special interest is the observation that aryl alkyl ketones (ArC(O)R) with sterically bulky alkyl groups (*e.g.*, R=*iso*-propyl, *tert*-butyl and *cyclo*-propyl) were enzymatically reduced for the first time to the corresponding alcohols with greater than 96% ee. It was also found that, while linear alkyl chains induced an enantiopreference reversal of the reduction, the enantiopreference remained unchanged and enantioselectivity was dramatically enhanced when the alkyl group became branched (Table 1) [11, 14].

Recently, Kamitori et al. reported the X-ray structures of this carbonyl reductase **SSCR** and its complex with a coenzyme, NADPH (PDB code=1Y1P), and provided an analysis of the active site for the reduction of ethyl-4-chloro-3-oxobutanoate [15]. By manually fitting substrates into the active site (followed by energy minimization) these researchers identified the hydrogen-bonding residues (Ser133, Thr134, Tyr177, and the C4 atom of the

Table 1 Asymmetric reduction of aryl alkyl ketones catalyzed by carbonyl reductase from *Sporobolomyces salmonicolor* (**SSCR**)



R	X	ee (%)	Absolute configuration
CH_3	H	42	R
CH_2CH_3	H	28	R
$(CH_2)_2CH_3$	H	88	S
$(CH_2)_3CH_3$	H	87	S
$(CH_2)_4CH_3$	H	34	S
$(CH_2)_5CH_3$	H	27	S
$CH(CH_3)_2$	H	98	R
$C(CH_3)_3$	H	98	R
cyclo-C_3H_5	H	96	R
cyclo-C_3H_5	F	98	R
cyclo-C_3H_5	Cl	98	R

nicotinamide ring of the NMN-AMP-PO_4 cofactor, NMN= nicotinamide mononucleotide, AMP= adenosine monophosphate) that "fix" the carbonyl oxygen in the active site, and a hydrophobic channel (mobile region Ile91-Tyr101, Phe97, Trp226, Ala238, Leu241, Pro170, and Leu174). The hydrophobic channel was proposed to not only play a role in allowing access to the active site, but also in substrate recognition. Given the intriguing enantiopreference patterns seen by Zhu et al. [14], a molecular modeling analysis of different aryl alkyl ketones in the 1Y1P active site has been performed to understand the enantioselectivity of aryl alkyl ketone reductions catalyzed by this NADPH-dependent carbonyl reductase at the molecular level.

Computational methods

The Molecular Operating Environment (MOE, version 2003.02) [16] program was utilized for all calculations. Molecular mechanics calculations utilized the Amber94 force field [17] and Marsilli-Gasteiger "Partial Equalization of Orbital Electronegativities" (PEOE) atomic charges [18].

Receptor site search

Potential receptor sites within 1Y1P were identified using the Site Finder functionality within MOE, which utilized a geometry-based (as opposed to an energy-based) technique, and which was based upon the alpha shape methodology [19, 20]. Operational settings employed in this research were as follows: Probe Radius 1=1.4 Å (radius of a hypothetical hydrophilic, hydrogen-bonding atom), Probe Radius 2=1.8 Å (radius of a hypothetical hydrophobic atom), isolated Donor/Acceptor=3 Å (if a hydrophilic alpha sphere had no hydrophobic alpha sphere within the specified distance, then the former was discarded; this minimized sites likely to bind only water), Connection Distance=2.5 Å (if individual clusters had two alpha spheres within this distance they were combined), Minimum Site Size=3 (minimum number of alpha spheres that comprised a suitable receptor site), Radius=2 Å (sites smaller than this size were eliminated). Solvent and salts were excluded in these simulations.

Docking simulations

Aryl alkyl ketones were docked in a 50 Å×50 Å×50 Å box that encompassed the two largest receptor sites found. The enzyme geometry was fixed, while the substrate geometry was flexible throughout these docking simulations. Water molecules in the vicinity of the receptor sites were deleted. To minimize the computational effort per run (and thus conduct more docking runs), a truncated receptor site model of 1Y1P was validated (residues included were those that had any atom within 7.5 Å of either of the two largest receptor sites or the cofactor). Test docking simulations with propiophenone using the full enzyme did not lead to appreciably different results versus those obtained with the truncated receptor site model, so that the latter model was employed for other ketones.

The Tabu search algorithm was employed for docking [21]. Tabu employed a list of previously visited conformations (in this case 100) to keep the search exploring novel areas of docking space. One hundred docking runs (each with a random starting geometry of substrate) were utilized. Each run entailed 1000 steps (maximum number of iterations in which the substrate was moved), and 100 attempts per step (maximum number of substrate conformations explored per step).

Geometry optimization of receptor site-substrate complex

The low energy substrate/receptor site conformations obtained from the docking simulations (*vide supra*) were first submitted to molecular dynamics (constant NVT, 300 K, 100 ps equilibration, 1 fs step size). The lowest energy conformations from the molecular dynamics simulations were then geometry optimized using the Amber94 force field [17]. To maintain the structural integrity of a full enzyme model, backbone atoms and the AMP and phosphate groups of the NMN-AMP-PO_4 cofactor were frozen during both the molecular dynamics simulations and subsequent geometry optimizations.

Results and discussion

In carrying out the analysis of aryl alkyl ketone binding, we proceeded in three phases. First, receptor sites in 1Y1P [15] were identified. Second, substrates were then docked into the receptor sites found in the first step. In this step, the substrate was flexible while the enzyme active site was fixed in geometry. The third step entailed molecular dynamics/energy minimization of receptor-substrate complexes for low energy docked conformations obtained from the second step.

Receptor sites in the NADPH-dependent carbonyl reductase (*S. salmonicolor*)

The structure of NADPH-dependent Carbonyl Reductase (*S. salmonicolor*) was downloaded from the Protein Data Bank [22] (PDB code=1Y1P [15]). Hydrogens were added to all atoms, taking care that the NMN unit of the cofactor was reduced at carbon-4. There are two molecules in the asymmetric unit of 1Y1P; as both units are nearly identical in geometry, only one of these monomer units was utilized in the subsequent calculations. The enzyme geometry was then optimized in two steps. With the heavy atom positions fixed at their crystallographic positions, hydrogen atom positions were geometry optimized. The enzyme geometry was then fully optimized. Both sets of calculations were performed with the Amber94 force field [17].

The optimized geometry of 1Y1P was analyzed using the Site Finder functionality in MOE [16, 19, 20]. Interestingly, the two largest receptor sites found by this automated routine are both in the vicinity of the NMN-AMP-PO_4 cofactor, as shown in Figs. 1 and 2. Indeed, given their proximity (note, for example, that they share residues Phe97, Leu174, Trp226 and Met242) these two active sites could be viewed as comprising a single, larger active site - the larger portion being catalytic in its function and the smaller being akin to the hydrophobic channel of Kamitori et al. [15]. Amino acid residues close to the largest (catalytic) receptor site (Fig. 1) are Val95, Phe97, Ser133, Thr134, Val135, Ile139, Pro140, Leu174, Tyr177, Pro206, Asn207, Tyr208, Gly221, Ser222, Thr223, Trp226, Met242, Pro243, and Gln245.

The second largest receptor site (hydrophobic channel) identified by MOE is very near to the one just discussed. Compared to the catalytic site in Fig. 1, this second receptor site (Fig. 2) is (a) further from the cofactor, (b) more hydrophobic than the largest receptor site, and (c) contains primarily the residues that comprise the hydrophobic channel [15]: Phe97, Pro170, Gln171, Ser173, Leu174, Trp226, Ser236, Ala238, Leu239, Leu241, Met242 (Fig. 2; viewed from the same reference point as Fig. 1). It should be noted that the designation of a hydrophobic/hydrophilic sites in Figs. 1 and 2 not only indicates a vacancy in the enzyme surrounded by hydrophobic/hydrophilic amino acid residues, but also further implies that those portions of the substrate with complementary chemical properties will bind preferentially in the corresponding sites. Hence, the Ar and R groups of the ketone substrate are expected to bind

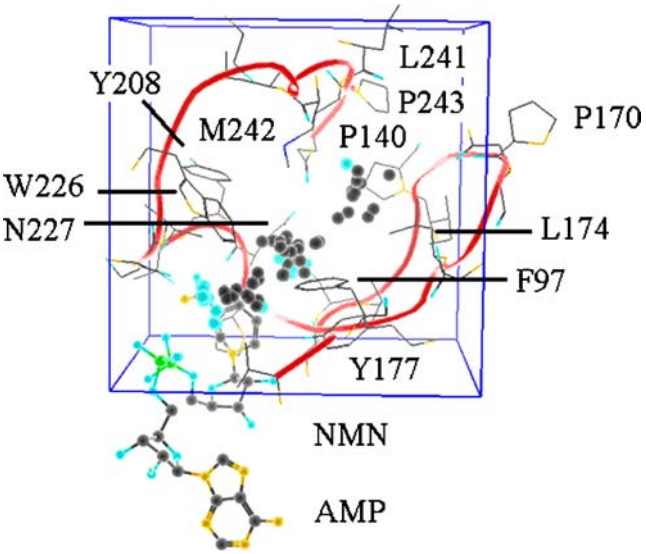


Fig. 1 Largest (catalytic) receptor site in NADPH-dependent carbonyl reductase (*S. salmonicolor*). Hydrogens omitted for clarity. Amino acid residues are shown in wire frame, NMN-AMP-PO_4 in ball-and-stick mode. Receptor sites are denoted as spheres (blue spheres for a hydrophilic vacancy; gray spheres for a hydrophobic vacancy)

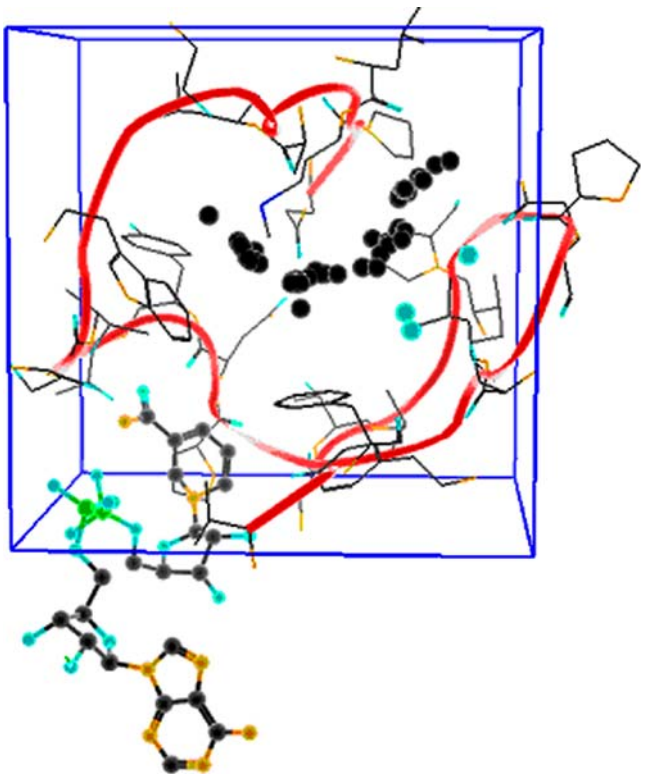


Fig. 2 Second largest receptor site in NADPH-dependent (**SSCR**). View is from the same perspective as in Fig. 1

preferentially within hydrophobic sites while the carbonyl group is predicted to seek out hydrophilic sites.

Ketone docking to receptor site models

Docking of aryl alkyl ketones to 1Y1P was performed using the Tabu algorithm [21] as outlined in computational methods; the enzyme receptor site geometry was fixed, while the substrate geometry was flexible throughout these docking simulations. The docking box fully enclosed the two largest receptor sites, and to minimize the computational effort a truncated receptor site model of 1Y1P was used (residues within 7.5 Å of either the two largest receptor sites or the cofactor).

Several interesting features were identified from the docking simulations. First, docking of the aryl alkyl ketones is energetically preferred in the catalytic portion of the combined catalytic/hydrophobic receptor site, i.e., the portion that is more hydrophilic and closer to the cofactor (Fig. 1). This is similar to the substrate-binding structure of camphorquinone which fits into the hydrophobic pocket, but distinct from that of ethyl 4-chloro-3-oxobutyrate in which the CH_2Cl group is placed in the hydrophobic pocket and $CH_2CO_2C_2H_5$ fits in the hydrophobic channel in the model proposed by Kamitori et al. [15]. Second, the favorable docking conformations in the catalytic site for the ketones typically fall into two ensembles, which are labeled as "right" and "left" to describe the disposition of the phenyl substituent as viewed from the cofactor. In both of these low-energy conformational ensembles the carbonyl oxygen is positioned proximal to the NMN ring of the cofactor in the hydrophilic portion of the catalytic site. The docked conformational ensembles are distinguished by the positions of the substituents, presenting either the *Si* or *Re*-face of the ketone to the nicotinamide reductant. Figure 3 shows an overlay of the lowest energy conformations obtained by docking aryl alkyl ketones to the truncated catalytic site of 1Y1P. The docked conformations involve not a simple rotation of the ketone about the C=O bond axis, but rather a rotation and displacement to accommodate the triangular shape of the receptor site.

It is important to stress that all ketones have low energy "left" *and* "right" docking conformations as shown in Fig. 4, which are consistent with experimental observations of facile switching in enantioselectivity with alkyl group. With the exception of heptanophenone (that will be discussed below), "down" conformations (Fig. 4), that place the carbonyl oxygen in the hydrophobic portion of the catalytic receptor site, are found to be either very high in energy via the docking simulations or are not found at all.

The most favorable docking geometries of three aryl alkyl ketones (PhC(O)R), where R=^{n}Hx, ^{c}Pr, and ^{n}Bu, are dissimilar to the other ketones studied, and are highlighted in Fig. 3. Heptanophenone ($PhC(O)C_6H_{13}$) is docked with the carbonyl oxygen distal from the NMN ring. The disparate docking conformation of heptanophenone is likely due to the inability to fit the long alkyl chain in the receptor site, which can be viewed as a scalene triangle with sides of approximately 8 Å by 9 Å by 13 Å (Fig. 4), and that is also narrow in width. The poor fit for heptanophenone in the receptor site is reflected in the docked conformation of the ketone, i.e., the *n*-hexyl substituent does not adopt the zig-zag conformation of the "free" compound as do ketones with shorter alkyl chains, but a constricted conformer that is 2.4 kcal mol^{-1} above the global minimum for free heptanophenone. The lowest energy docked conformations

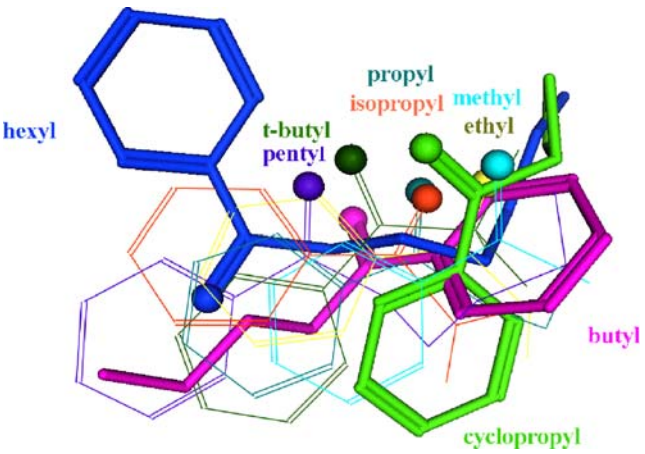


Fig. 3 Overlay of lowest energy docked conformations of aryl ketones (flexible) to truncated catalytic site (fixed geometry). Those with atypical docking conformations (R=^{n}Bu, ^{n}Hx, ^{c}Pr) are highlighted. Carbonyl oxygen atoms are denoted by a balls

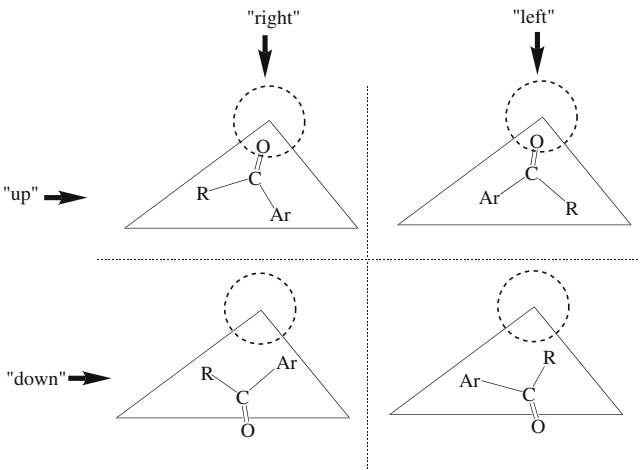


Fig. 4 Model of the catalytic portion of the receptor site (Fig. 1) viewed from "above" (i.e., from the cofactor). The dashed circle denotes the hydrophilic portion of the receptor site; hydrophobic residues dominate the remainder of receptor site. "Up" and "down" conformations are those in which the ketone oxygen is proximal and distal, respectively, with respect to the hydrophilic portion of the catalytic site

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^{-1}) "left" conformer	$E_{int.}$ (kcal mol^{-1}) "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 Table 1 for the definition of R and X. The enantiomeric excess is given in percent

of the cylopropyl and *n*-butyl substituted ketones are those with the phenyl ring on the "right" side of the receptor site, while others are docked with the phenyl ring on the "left" side of the receptor site (see Fig. 4 for a depiction of "left" and "right" descriptors).

The increase in energy that the docked propiophenone undergoes to accommodate binding in the receptor site is calculated to be very small, ~0.5 kcal mol^{-1} (recall that the enzyme is fixed in the docking simulations), less than half of the distortion energy of larger ketones. This result suggests that propiophenone is loosely bound in the receptor site, which may be reflected in its low ee% (28%, R>S) for reduction. The low ee% seen for acetophenone (42%, R>S) has a similar origin. The low ee% for heptanophenone (27%, S>R) may originate from its different docked coordination mode. The origin of the experimentally observed low ee% for hexanophenone (34%, S>R) is not clear from the simulations.

Receptor site/substrate complexes

The substrate is flexible (i.e., conformations resulting from torsions about rotatable bonds are permitted during the docking runs) in the docking simulations, but the enzyme is fixed in geometry. To enhance the simulations, the low energy docked conformations were submitted to molecular dynamics in order to allow the geometry to relax, and the resulting receptor-substrate geometries were fully optimized with the Amber94 force field [17].

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}) for the substrates with the active site were calculated using the Amber94 force field (Table 2). Interaction energies for the ketones are ≈ −20 kcal mol^{-1}, increasing slightly with the size of the alkyl group of the ketone. As expected, the simulations suggest that the majority (~85%) of the stabilization energy of the ketone in the receptor site is due to hydrophobic/van der Waals forces with a lesser contribution from electrostatic interactions.

From Table 2 it can be seen that, the interaction energies between the "right" conformer and the enzyme active site are lower than that of the "left" conformer for ketones PhC(O)R, where R=*n*-propyl, *n*-butyl, *n*-pentyl and *n*-hexyl. In the "right" conformer, the hydride is transferred from the cofactor to the carbonyl group at the *Re*-face, leading to the (S)-enantiomer. This is consistent with the experimental observation that the (S)-enantiomer was obtained as the major product. Conversely, for the other aryl alkyl ketones in Table 2, the "left" conformers have lower interaction energies with the active site. In these cases the reduction of these ketones gave (R)-enantiomer as the major product. Therefore, there is a correlation between the experimentally observed major enantiomer and the conformer having the most stable interaction energy with the receptor site (E_{int}).

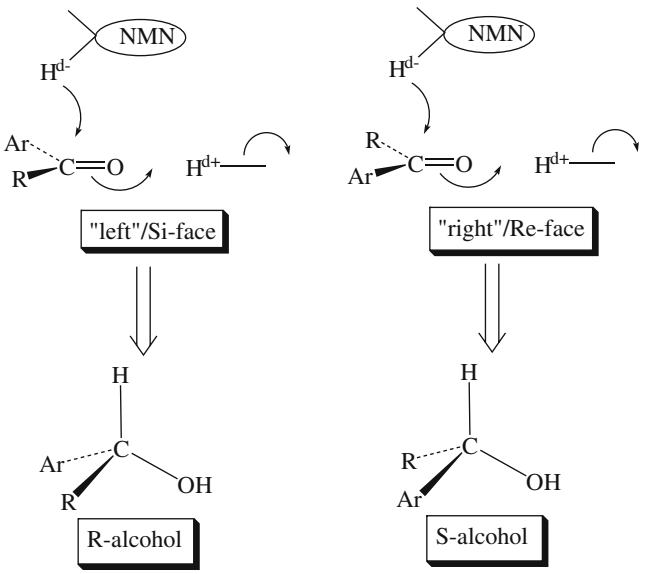


Fig. 5 Receptor site model for NADPH-dependent carbonyl reductase

Summary and conclusions

Automated structural analysis of *Sporobolomyces salmonicolor* carbonyl reductase [15], PDB code=1Y1P, indicates that the two largest receptor sites are in the vicinity of the nicotinamide reductant. Given their proximity, we view these as comprising a single, larger active site - one portion being catalytic in its function (Fig. 1) and the other (Fig. 2) is the hydrophobic channel that permits access to the catalytic site [15]. The catalytic portion of the receptor site (Fig. 1) is a scalene triangle with sides of approximately 8 Å by 9 Å by 13 Å (Fig. 4), and is narrow in width. This catalytic site has a corner that is surrounded by residues with hydrophilic side chains and/or side chains (Fig. 3) that could potentially hydrogen bond to the carbonyl oxygen, e.g., Ser133, Thr134, Tyr177, Asn207, Tyr208, Gly221, Ser222, Thr223, and Gln245. The hydrophobic site (Fig. 2) is more hydrophobic than the largest receptor site and is reminiscent of the channel identified by Kamitori and coworkers in their crystal structure analysis of 1Y1P [15].

Docking the aryl ketones to the receptor sites shows a distinct preference for binding to the catalytic receptor site (Fig. 1), and for conformations that place the carbonyl oxygen of the substrate proximal to the hydrophilic "corner" of the largest receptor site, so-called "up" conformations (Fig. 4). Favorable docking conformations for the aryl alkyl ketones studied typically fall into two low-energy ensembles: "right" and "left." These conformational ensembles are distinguished by the positions of the substituents, presenting either the *Si*-("left") or *Re*-("right") face of the ketone to the nicotinamide reductant (Fig. 4).

Low energy docked conformations of ketones were then submitted to molecular dynamics to allow the geometry to relax, and the resulting enzyme-substrate geometries were fully optimized. For the ketones investigated here, there is a correspondence between the experimentally observed major enantiomer of the alcohol obtained from **SSCR**-catalyzed ketone reduction and the conformer found to have the most stable interaction energy with the receptor site (E_{int}) in all cases: "left" → R-alcohol, "right" → S-alcohol (Table 2).

The receptor site modeling, docking simulations, and receptor site geometry optimizations lead to a straightforward model for understanding the enantioselectivity of these NADPH-dependent carbonyl reductases. The model is summarized in Fig. 5. When the ketone is bound preferentially in the "left" conformer, it presents the *Si*-face to the nicotinamide, which is the source of hydride. Conversely, the "right" conformer for ArC(=O)R presents the *Re*-face to the nicotinamide reductant. In both cases the carbonyl oxygen is hydrogen-bonded to residue Tyr177 in the hydrophilic portion of the receptor site, which can provide a proton to complete the reduction of the carbonyl to the alcohol. The receptor site model summarized in Fig. 5 not only rationalizes the observed stereospecificity of **SSCR** reductions of aryl alkyl ketones, but also provides a starting point for identifying point mutations to active site residues that can enhance selectivity and activity, as well as broaden the scope of possible substrates.

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