JOC Notes

Enantioselective Enzymatic Reductions of Sterically Bulky Aryl Alkyl Ketones Catalyzed by a NADPH-Dependent Carbonyl Reductase

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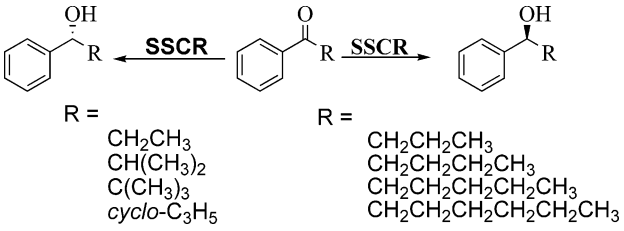

The enantioselective reductions of aryl alkyl ketones, ArC(O)R, with a diverse number of alkyl groups have been achieved with an isolated carbonyl reductase from *Sporobolomyces salmonicolor*. Of special interest is the observation that ketones with sterically bulky alkyl groups could be reduced to the corresponding alcohols in excellent optical purity. An unusual alkyl chain-induced enantiopreference reversal was observed but was shown to be consistent with the enzyme−substrate docking calculations.

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 protocols have attracted more and more attention.[1] Up to now, with the exception of the α- and *β*-ketoesters, most ketone reduction studies have been limited to those in which the ketones possess at least one small group such as methyl or halomethyl.[2−5] The reductions of aryl alkyl ketones with larger alkyl groups using isolated carbonyl reductase enzymes have scarcely been studied.[6−8] An alcohol dehydrogenase from *Pseudomonas sp.* has been evaluated toward the reduction of a few aryl alkyl ketones other than acetophenone.[8] However, enantioselectivity studies have only been reported for cyclopropyl phenyl ketone and 2-(1-pentanoyl)furan, with ee values being 92% and 45%, and the corresponding yields being 41% and 5−10%, respectively.[8] The enantioselective reduction of aryl alkyl ketones with sterically bulky alkyl groups, such as *tert*-butyl and isopropyl, still represents a significant challenge in both the enzymatic and chemical reductions of ketones.[9]

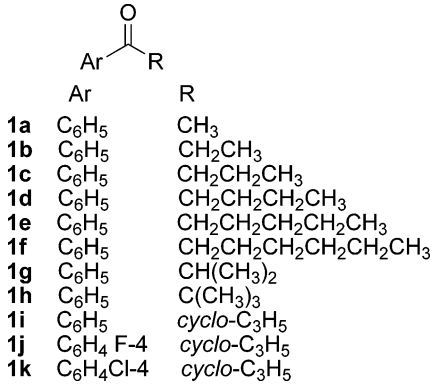

FIGURE 1. Aryl alkyl ketones.

Recently, a carbonyl reductase gene from red yeast *Sporobolomyces salmonicolor* AKU 4429 has been cloned and overexpressed in *E. coli*, and the encoded protein (**SSCR**) showed activity toward the reduction of various ketones, including aromatic and aliphatic ketones, as well as α- and *β*-ketoesters.[10,11] It was found that bulky α-ketoesters, such as ethyl 3,3-dimethyl-2-oxobutyrate, could be reduced to the chiral alcohols in very high ee values and yields.[11] Thus it was reasoned that **SSCR** might catalyze the enantioselective reduction of aryl alkyl ketones possessing sterically bulky alkyl groups. Therefore, we have studied the enzymatic reduction of a number of aryl alkyl ketones catalyzed by this NADPH-dependent carbonyl reductase (**SSCR)** (see Figure 1). The enzyme was found to catalyze the enantioselective reduction of sterically bulky aryl alkyl ketones, such as 2,2-dimethylpro piophenone, to give the corresponding chiral alcohols in high optical purity. An interesting alkyl chain-induced enantiopreference flip-flop was also observed in these studies and an enzyme−substrate docking analysis was performed to understand this observation at the molecular level.[12]

The carbonyl reductase gene (**SSCR**) from *Sporobolomyces salmonicolor* AKU4429 was cloned and overexpressed in *E coli*.

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TABLE 1. Asymmetric Reduction of Aryl Alkyl Ketones Catalyzed by Carbonyl Reductase (SSCR) from *Sporobolomyces salmonicolor*

ketones	specific activity[a]	ee (%)	absolute conf
1a	28	42	R
1b	48	28	R
1c	57	88	S
1d	114	87	S
1e	91	34	S
1f	45	27	S
1g	44	98	R
1h	201	98	R
1i	574	96	R
1j	303	98	R
1k	271	98	R

[a] The specific activity was defined in units of nmol•min^{-1}•mg^{-1}.

The encoded protein was then purified using literature procedures.[10,11] The specific activity of carbonyl reductase **SSCR** toward the reduction of a series of 1-phenylalkanones was determined by spectrophotometrically measuring the oxidation of NADPH at 340 nm at room temperature as previously described.[11] Activity assay with the control cell-free extract, which was prepared by the expression of the pET15b vector without the **SSCR** gene in *E. coli* BL21(DE3) strain, did not show activity toward the substrates. The enantioselectivity of the carbonyl reductase was studied by using a NADPH regeneration system consisting of D-glucose dehydrogenase (**GDH**) and D-glucose.[11,13] The enantiomeric excess (ee) values of the product alcohols were determined by chiral GC analysis. The absolute configurations of the product alcohols were assigned by comparing either their retention times with standard samples[14] or their specific rotation with reported data.[9,15] The results are summarized in Table 1.

As can be seen from Table 1, the carbonyl reductase (**SSCR**) effectively catalyzed the reduction of all the aryl alkyl ketones shown in Figure 1. The activity of this enzyme first increased as the alkyl chain became longer (**1a** → **1d**), reaching its highest activity when R = *n*-butyl (**1d**). However, the enzyme became less active when the alkyl chain length was further increased (longer than 4 carbons, **1e** and **1f**). Surprisingly, **SSCR** showed a higher activity toward the reduction of ketones with bulky alkyl groups, such as the *tert*-butyl group (**1h**). Indeed, compound **1h** showed a higher activity than those found for the smaller alkyl groups (**1a**−**c**,**g**) and its linear counterpart (**1d**). Among all the tested aryl alkyl ketones, the carbonyl reductase was most active toward the reduction of the aryl cyclopropyl ketones (**1i**−**k**).

An intriguing phenomenon was observed for the enantioselectivity of **SSCR**-catalyzed reductions of the aryl alkyl ketones. The reduction of acetophenone (**1a**) and propiophenone (**1b**) produced the (R)-enantiomer as the major product, while the (S)-enantiomers were obtained in 87−88% ee when the alkyl group was *n*-propyl (**1c**) or *n*-butyl (**1d**). The enantioselectivity decreased as the alkyl group became longer (C_5 and C_6 chain), but (S)-configuration alcohols were still the major products. Interestingly, when the alkyl group became branched, for example, isopropyl (**1g**), cyclopropyl (**1i**), or *tert*-butyl (**1h**), the enantiopreference did not reverse, but afforded the (R)-enantiomers with dramatically higher enantioselectivity (96−98% ee) than those found for the methyl (**1a**) or ethyl (**1b**) groups. This is remarkable in that it is in contrast to the usual observation that the enantioselectivity decreases when the R group becomes branched.[16] In general, high enantioselectivity is difficult to achieve in the reduction of ketones with bulky groups via metal-catalyzed hydrogen transfer or hydrogenation.[9,17,18] The reductions of butyrophenone (**1c**), 2,2-dimethylpropiophenone (**1h**), and phenyl cyclopropyl ketone (**1i**) were carried out on a millimole scale and the corresponding chiral alcohols were isolated in greater than 90% yields with high optical purity, demonstrating the applicability of carbonyl reductase **SSCR** in the preparation of these chiral alcohols.

A similar substrate-size-induced reversal of stereoselectivity has previously been observed for the reduction of methyl alkyl ketones (CH_3COR, R is alkyl) catalyzed by an alcohol dehydrogenase from *Thermoanaerobium brockii*.[19] Since the X-ray structures of this carbonyl reductase **SSCR** and its complex with a coenzyme, NADPH, have been determined,[10,20] an enzyme−substrate docking analysis was performed on the **SSCR** protein structure (PDB file 1Y1P) wth the FlexX program. Such an analysis would be useful in our understanding of the alkyl chain induced enantiopreference reversal at the molecular level. Three residues (Ser133, Try177, and Lys181) were proposed as the catalytic triad in this short-chain dehydrogenase/reductase enzyme. The carbonyl oxygen atom of the substrate formed hydrogen bonds with the Ser133 and Tyr177 residues, and was protonated from the Tyr177 residue, followed by the attack of a hydride from the C4 atom of NADPH at the carbonyl carbon atom of the substrate.[20,21] Therefore, care was taken to orient the hydrogens of hydroxyl groups of Ser133 and Tyr177 toward the position of water molecule HOH175, which the carbonyl oxygen of the substrate would occupy during docking. The overlay of the lowest energy docked conformations of propiophenone (**1b**) and butyrophenone (**1c**) into the enzyme active site are shown in Figure 2 (for individual docking pictures, see the Supporting Information). In both lowest energy conformational ensembles the carbonyl carbons were in a position proximal to the C4 atom of the nicotinamide ring of the cofactor NADPH (3.311 and 3.394 Å for propiophenone (**1b**) and butyrophenone (**1c**), respectively). The ensembles differed from one another by the orientations of the substrates at the enzyme active site. The nicotinamide ring of the cofactor was located at the *Si*-face of propiophenone, but at the *Re*-face of butyrophenone. Thus these ketones should be reduced to (*R*)-1-phenylpropanol and (*S*)-1-phenylbutanol, respectively, which is consistent with our experimental observations. The lowest energy docked conformation of 2-methylpropiophenone (**1g**) showed that the nicotinamide ring of NADPH was located at

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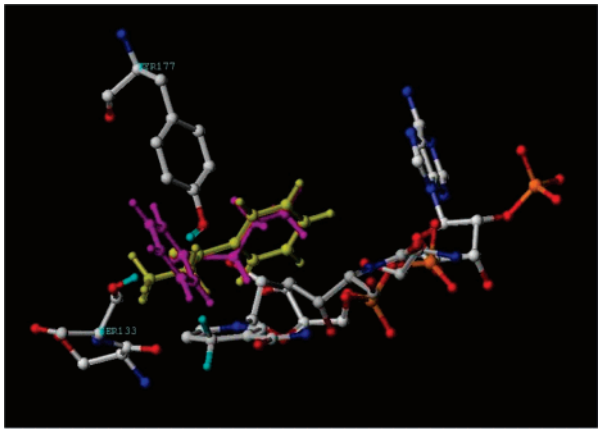

FIGURE 2. The overlay of lowest energy docked conformations of propiophenone (golden yellow) and butyrophenone (purple) into the enzyme active site.

the *Si*-face of substrate (see the Supporting Information), leading to (*R*)-2-methyl-1-phenylpropanol. The enzyme−substrate docking of the other aryl alkyl ketones also showed the preferred docking conformations to be consistent with the experimental results.

In summary, a carbonyl reductase from *Sporobolomyces salmonicolor* (**SSCR**) was found to effectively catalyze the enantioselective reduction of aryl alkyl ketones having a number of diverse alkyl groups. Significantly, ketones with sterically bulky alkyl groups such as isopropyl, *tert*-butyl, and cyclopropyl were enzymatically reduced for the first time to the corresponding alcohols with greater than 96% ee. It was also found that, while a linear alkyl chain induced an enantiopreference reversal of the reduction, the enantiopreference remained unchanged and enantioselectivity was dramatically enhanced when the alkyl group became branched. The lowest energy conformations of the aryl alkyl ketones in the enzyme active site, obtained from enzyme−substrate docking studies, were found to be consistent with these intriguing experimental results. Thus, enzyme−substrate docking analyses may provide useful guidance in the rational design of new carbonyl reductases with desired enantioselectivity. Such studies are currently underway in our laboratories.

Experimental Section

Preparative Scale Ketone Reduction. The preparative syntheses were carried out as follows: d-glucose (1.0 g), D-glucose dehydrogenase (10 mg), NADPH (10 mg), and carbonyl reductase **SSCR** (10 mg) were mixed in a potassium phosphate buffer (50 mL, 100 mM, pH 6.5). A solution of the aryl alkyl ketone (500 mg in 2.0 mL of DMSO) was added to this mixture that was then stirred at room temperature until conversion was complete (usually overnight). During the reaction the pH was controlled at 6.5−6.6 with the addition of a 0.5 M NaOH solution. After reaction, the mixture was extracted with methyl *tert*-butyl ether and the organic extract was dried over anhydrous sodium sulfate. Removal of the solvent gave the particular alcohol product, which was identified by comparison of ^{1}H and ^{13}C NMR with literature data.[9,16,18,22] (*S*)-1-Phenylbutanol (**2c**): 466 mg (93% yield); $[\alpha]^{22}_{D}$ −38.1 (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]^{28}_{D}$ −47.9 (*c* 0.34, benzene)). (*R*)-2,2-Dimethyl-1-phenylpropanol (**2h**): 475 mg (95% yield); $[\alpha]^{22}_{D}$ +30.4 (*c* 1.0, $CHCl_3$) (lit.[9] $[\alpha]^{25}_{D}$ +24.3 (*c* 2.2, benzene)). (*R*)-Cyclopropylphenylmethanol (**2i**): 460 mg (92% yield); $[\alpha]^{22}_{D}$ −32.4 (*c* 1.0, $CHCl_3$) (lit.[15] $[\alpha]^{28}_{D}$ +22.6 (*c* 0.234, $CHCl_3$)) for *S*-enantiomer (90% ee).

Enzyme−Substrate Docking Studies. The crystal structure of the SSCR/NADPH (PDB code 1Y1P) was used in the calculations. In the crystal structure, two nearly identical protein molecules (Mol-1 and Mol-2) were found. The docking was performed on Mol-1 where an NADPH was tightly bound. Since the carbonyl oxygen atom of the substrate was assumed to form hydrogen bonds with Ser133 and Tyr177, the torsion angles of Ser133 and Tyr177 were adjusted so that the hydrogens of their hydroxyl groups were oriented toward the water molecule (HOH175) in the crystal structure, which was coincident with the carbonyl oxygen of the docked substrate. All docking calculations were performed with the FlexX program in the SYBYL7.1 modeling package. A docking algorithm that took into account ligand flexibility but kept the protein rigid was employed. All docking runs were carried out with the standard parameters of the program for interactive growing and subsequent scoring.

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Supporting Information Available: General methods, procedures for activity assay and ketone reductions, details of chiral GC, and ^{13}C NMR spectra for the alcohols obtained in the preparative scale ketone reductions. This material is available free of charge via the Internet at http://pubs.acs.org.

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